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AQUATEC BIOLOGICAL SCIENCES

AQUATEC BIOLOGICAL SCIENCES, INC.

QUALITY ASSURANCE PROGRAM PLAN

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Revision 9

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1.0 QUALITY ASSURANCE POLICY STATEMENT

Policy

Aquatec Biological Sciences (Aquatec) shall provide testing services that:

- Conform to our clients needs, requirements, and intended use,
- Comply with applicable government standards and regulations,
- Comply with current laboratory accreditation standards, including the State of New Hampshire Department of Environmental Services Environmental Laboratory Accreditation Program (NH ELAP) as defined in the National Environmental Laboratory Accreditation Conference (NELAC) standards.

Continuous efforts toward improvement are integral to laboratory activities. These improvements ensure that Aquatec maintains the high standards defined by NELAC and other regulatory performance requirements.

Purpose

The purpose of this manual is to establish the framework and to assign responsibilities to ensure that all employees conform to the policy. Additionally, this manual is used to inform appropriate regulatory authorities and clients of the quality practices in place at Aquatec. This Quality Assurance Program Plan (QAPP) formalizes the Quality System that has been established and that is in operation for all sample-related activities including employee training, sample handling, sample preparation, sample analysis, and documentation.

Scope

This QAPP applies to all employees. There is a firm commitment from all members of this laboratory to follow a comprehensive Quality Assurance Program Plan. This commitment and dedication to quality is fully supported from the bench level to upper management in order to meet the objectives of our laboratory and best serve our clients.

This QAPP undergoes an annual review by the Director and Department Managers. Revisions to the QAPP are distributed throughout the laboratory to replace the outdated copies so that only the most current revision is available to clients. It is the responsibility of the Director to ensure that all employees familiarize themselves with and comply with the procedures presented in this manual and associated documentation.

The policies and practices of quality assurance/quality control presented in the following text are set forth as minimums. Reading of the most current version of the QAPP and a companion document, The Employee Manual is required of employees. Formal, signature page documentation of reading and understanding the QAPP (and annual revisions of the QAPP) is required.

2.0 FACILITY DESCRIPTION AND CAPITAL EQUIPMENT

2.1 LABORATORY FACILITIES

Aquatec has been active in environmental investigations and sample analysis since it's founding in 1996. Aquatec is located at 273 Commerce Street, Williston, VT.

The floor plan in Appendix E (Figure 1) shows the location, size, and utilities available. Aquatec has the support systems for biological testing and analysis: reverse osmosis and organic removal (carbon) and deionizing systems, computer networking, controlled-temperature laboratories and systems, temperature and water quality monitoring equipment, microscopes, drying oven, incubators, boats, field sampling equipment, and other equipment associated with microbiological, toxicological, and ecological tests or investigations.

2.2 SECURITY

2.2.1 Laboratory Security

Employees are instructed in Aquatec's security policies (e.g., sample security, door policies, data filing, etc.). Because of the nature of our work, security of the facilities, equipment, and project files is necessary. Access to the laboratories on a daily basis is limited to employees. Visitors, regulatory personnel, or clients are greeted at the entrance. If access to laboratory spaces by the visitor is warranted, an employee will accompany the visitor into those spaces until the time that the visitor departs Aquatec. Access doors to Aquatec are maintained in a locked mode during non-business hours.

2.2.2 Data Security

Aquatec employees are expected to be familiar with and adhere to standards of confidentiality mandated by individual contracts and common sense business practices. These confidentiality procedures may include signing project-specific confidentiality agreements and obtaining client permission to release information in other forums (This may exclude those data required to be released through court orders or other legally-binding measures). Administrative actions for possible breaches in confidentiality are outlined in the Employee Manual (Section 8).

2.2.3 Computer Security

Computer access to utilization of specific PC's are password-protected. Sensitive data and data files are stored on our Dell server. These files have various levels of security protection dependent upon the nature of information. Files that are routinely available for use by authorized personnel have the broadest level of security- read/write access. Files that are necessary for communication of information are more restricted-access

for read only provided to those authorized in the workgroup. The most restrictive files do not have share access and aren't available to be listed by non-authorized individuals.

The file sharing security is dictated and managed by the department manager when routine and communication files are needed. The restricted access files are dictated and managed by the Director. The server is password protected and therefore unauthorized access directly through physically logging onto the server is minimized. Administrative actions for possible breaches in confidentiality are outlined in the Employee Manual (Section 8).

2.3 EQUIPMENT AND REAGENT INVENTORY

2.3.1 Laboratory Equipment

An equipment list for Aquatec Biological Sciences, Inc. is located in Appendix F.

Equipment and reference standards (certified thermometers and reference weights) in the Toxicology and Microbiology Laboratories must be properly maintained, inspected, and cleaned. Maintenance procedures must be documented. A Master List of Equipment will be maintained. The Master List must include the following information for each major item of equipment:

- Description of equipment, Model Number, serial number (if provided)
- Manufacturer
- Date received
- Date placed in service
- Current storage or use location
- Condition (e.g., new, used, reconditioned)
- Date and description of maintenance procedures
- History of damage, malfunction, modification, or repair

The records for the above are maintained in the Master Equipment Data Base on the server. This record should be updated as new equipment is acquired or existing equipment is maintained, repaired or taken out of service. Use the Equipment Maintenance Form (Appendix E) to document changes in the status of equipment or record the information in the Equipment Log, stored at the front desk.

Records for instruments used and calibrated frequently or on a daily basis are maintained in the Calibration Logbook.

2.3.2 Reagents

Reagents (standards for calibrations or DMR standards) are chemical and or biological products used to prepare lab waters, reference toxicants, media, or other solutions or

laboratory reagents. These are tracked through the Reagent Log and also the Reagent Preparation Log. The package is opened and the contents examined. If any products are received in a damaged condition, this is noted in the Reagent Log.

The date of receipt and initials of the person receiving the package, along with the vendor, description of product, and vendor lot number (if supplied) are entered into the Reagent Log. An Aquatec Lot # is assigned. This can be the same as the vendor lot # or it can be a created lot #. The Aquatec Lot # should follow the product throughout its use at Aquatec. This number is used in the Reagent Preparation Log when any reagents are prepared using this product. When the product is depleted (empty bottle) or expired, the discard date / initials should be entered in the final column of the reagent log.

2.3.3 Refrigeration Systems

The following is a list of refrigerators available for cold storage of samples:

Make and Model	Description	Quantity
Hobart	Three door refrigerator units	2
Hobart	One door freezer unit with Max/Min Thermometers	1

2.3.3 Information Systems

Aquatec's computer system is a server-based peer-to-peer network with a Dell PowerEdge 2500 SC server as the storage backbone. This server has three partitioned hard drives with a RAID 5 operating system for backup redundancy to minimize the risk of data loss. Each office and Sample Management has at least one Dell Dimension PC. Access to the LIMS system and project files is controlled through individual user accounts and passwords. Other hardware includes integrated high-speed printers, scanner, and fax machine for preparing hard copies of data and reports.

Most of the data handling, analysis, and reporting are completed using commercially available software. Our proprietary LMS system uses a Microsoft ACCESS platform while other specialized needs are accomplished using Microsoft Word and/or Microsoft Excel. Statistical analysis of environmental toxicology data is generally performed using TOXIS2 and TOXSTAT statistical packages.

3.0 PREVENTIVE MAINTENANCE OF LABORATORY EQUIPMENT

3.1 ROUTINE MAINTENANCE

Aquatec employs preventive maintenance to ensure that instrumentation is operating

within normal ranges and also to prevent equipment down time and to help ensure data validity to the best of our ability. General preventive maintenance procedures, many of which are unique to particular instruments, are outlined in each instrument's operation manual and/or laboratory standard operating procedures (SOPs). These documents also assist in the identification of commonly needed replacement parts, so that a supply of these parts can be maintained at the laboratory. It is the Department Manager's responsibility to make sure that the most current operation manuals and SOPs are available to analysts in the laboratory. Analysts perform documented routine maintenance of instrumentation while external technicians may be called in for major repairs or annual maintenance (e.g., Mettler balance annual calibration).

A bound calibration and maintenance log notebook is maintained to record routine and non-routine maintenance performed on instruments.

3.2 PREVENTIVE MAINTENANCE OR CALIBRATION CHECKS FOR EQUIPMENT

Balances are serviced by an external certified service engineer on an annual basis. Thermometers and temperature probes are calibrated at least annually against an NIST traceable reference thermometer, typically in the range-of-use. Records of external or internal calibrations are maintained.

The following instrument preparations or calibrations are performed and documented by analysts:

- a) Analytical balances are checked daily or at the time of use with class S weights. This check is documented in the Instrument Calibration Logbook.
- b) The daily temperature readings of the ovens, refrigerators, incubators and other temperature-controlled equipment are automatically logged (Scanlink System) and/or manually checked and recorded.
- c) Daily calibrations and/or routine maintenance of supporting laboratory equipment (e.g., meters and probes used for water quality measurements) are documented.
- d) Annual calibration of temperature probes and thermometers is required. To meet NELAC standards, each thermometer is required to have an identifying tag, date of calibration check, and correction factor. The annual calibration, by comparison to NIST traceable references, must be documented.
- e) Documented calibration checks of new volumetric vessels, adjustable pipettes, or lab-constructed volumetric containers is required. Records of pipette calibrations are maintained in the Calibration Log.
- f) Documented quarterly verification of light-cycle function is required. Records of light cycle verifications are maintained in the Calibration Log.

Any discrepancies are brought to the immediate attention of the Laboratory Manager and/or Director.

4.0 ADMINISTRATIVE ORGANIZATION

4.1 PERSONNEL QUALIFICATIONS

4.1.1 Minimum Requirements

Resumes of our key staff and experience and educational profiles can be found in Appendix A.

4.1.1.1 Director / Quality Assurance Officer

The Laboratory Director may also be the acting Quality Assurance Officer. The Laboratory Director / Quality Assurance Officer must hold a M.S. or higher degree in Biological Sciences and have a minimum of five years experience in environmental biology (three years of academic training past the M.S. level can be applied to the experience requirement). The Director should also have leadership ability, communications skills, and the ability maintain and improve laboratory operations.

4.1.1.2 Department Managers

Department Managers must, as a minimum, hold a Bachelor's Degree in a relevant scientific field of study (e.g., biology, microbiology, ecology, environmental biology, environmental chemistry). In addition, Department Managers must have at least five years of experience in the area of responsibility and be knowledgeable regarding applicable regulatory requirements and published protocols. Department Managers should also have leadership ability, communications skills, and the ability maintain and improve laboratory operations.

4.1.1.3 Laboratory Analysts

A Laboratory Analyst is defined as an employee responsible for generating or recording scientific data from on-going tests or investigations. Full-time Laboratory Analysts have, as a minimum, a Bachelor's Degree in a relevant scientific field of study. Laboratory Analysts must complete the training requirements outlined in Section 4.3. Requirements for performing specific laboratory skills (e.g., washing glassware, using a balance, colony counting, instrument use, or working on toxicity tests) are outlined in the pertinent SOPs for the specific tasks. Documentation of reading, demonstration of understanding, and agreement to follow the procedures outlined in SOPs is incorporated into our training program for Laboratory Analysts.

4.1.1.4 Laboratory Technicians

Part-time or temporary Laboratory Technicians must have completed High School have one year experience in the laboratory section where they are working or had one or more college courses in the biological sciences with emphasis on a relevant field of study. Part time and temporary analysts must work under the direction of laboratory manager or qualified laboratory analysts.

4.1.2 Personnel Documentation

Personnel files will be maintained in the Administration Office for all employees. Upon hiring, files will be started for the new employee and remain confidential with access by administrative personnel only. Outside audits will be permitted to view only the technical information as needed for audit purposes unless specific written permission is given by the technical director. The initial filing of documents will include the following:

Initial Documentation

Technical (Available to the Director and Toxicology Lab Manager)

1. Resume and any training certificates earned
2. Signed acceptance of Aquatec's QAPP
3. Signed acceptance of Aquatec's Chemical Hygiene Plan
4. Signed acceptance of Aquatec's Employee Manual
5. Signed recognition of the ethical and legal responsibilities and potential punishment (as included in the Employee Manual)

Administrative (Available to the Director and Administrative Assistant)

1. Copy of employment offer
2. Payroll Information
3. Health Enrollment Forms

Ongoing Documentation (Available to the Director and Toxicology Lab Manager)

Technical

1. Annual Updates of Resumes
2. Training Certificates and/or other training documentation
3. Signed acceptance of revisions of QAPP, SOPs, Etc.
4. SOP proficiency documentation
5. Annual QA proficiency demonstrations
6. Performance Reviews

Administrative (Available to the Director and Toxicology Lab Manager)

1. Personnel Review
2. Updates of payroll and health information
3. Letter of termination and exit interview

4.2 ROLES AND RESPONSIBILITIES

4.2.1 Director / Quality Assurance Officer

The Director is ultimately responsible for ensuring data quality and providing technical direction at Aquatec. The Director / Quality Assurance Officer develops policies and general quality assurance strategies in collaboration with the Department Managers. Additionally, the Director is responsible for reviewing all new incoming work and making sure that the appropriate facilities and resources are available before commencing such work. The Director is also responsible for general management of the laboratory, scheduling and execution of testing, and release of testing data and results. Any discrepancies in methodology, procedures, QC criteria, or reporting will be communicated to the Director. When Managers detect discrepancies or problems, they are reported to the Director / Quality Assurance Officer who in conjunction Managers is responsible for developing a corrective action procedure. The Director will also review and provide technical and statistical insight and acceptance/rejection criteria for scientific experimentation that does not have regulatory protocol-driven criteria.

The Director / QA Officer is responsible for the preparation and maintenance of the QAPP, and will assist Department Managers in the preparation, revision, and distribution of SOPs. The Director / QA Officer acts as the official laboratory contact for audits, performance evaluation studies and project-specific quality control issues. The Director / Quality Assurance Officer approves and confirms the implementation of documented corrective actions for events of non-compliance and also performs internal audits.

The Director / QA Officer is responsible for developing acceptance / rejection criteria for procedures where no method or regulatory criteria or published guidance exist. In cases where no method or regulatory criteria or published guidance are available, the Laboratory Director or designee will communicate with the client to determine the overall objectives of the study. He will also use his best professional judgment in combination with the defined scope of work to develop acceptance / rejection criteria *a priori* to initiating the study. In certain experimental investigations it may not be possible to defined exact “control” acceptability criteria, however as a minimum, the experimental environmental conditions should be within a range supporting any the testing conducted.

The Director will also ensure that all staff members are made aware of their respective designated responsibilities and that they are fully aware of the extent and limitations of their responsibilities.

The Director will afford clients (or their approved representatives) opportunities to clarify their requests or work plans and will monitor laboratory performance in relation to the work performed.

4.2.2 Department Managers

Department Managers are responsible for the training and daily operations of laboratory staff. Training of staff includes documented reading of the QAPP, Employee Manual,

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and documented reading of relevant SOPs. Laboratory personnel are expected to have a working knowledge of the Quality Assurance Program Plan (QAPP). Copies of the most current edition of the QAPP and SOPs are made available to each employee for reading and for signature.

Department Managers are responsible for the flow of work and data produced in their laboratories. Department Managers report directly to the Director. Each Department Manager has analysts who report directly to them. Department Managers are responsible for the maintenance of SOPs and the distribution and enforcement of the QAPP and SOPs in their laboratory sections. Discrepancies in Quality Control (QC) criteria will be brought to their attention, and a decision will be reached as to whether or not the data are acceptable. If, in their judgment, there are technical reasons which warrant the acceptance of what appears to be out-of-control data, these reasons must be documented and discussed with the Director / Quality Assurance Officer before the sample data are reported.

Laboratory Managers assign laboratory analysts for lab operations and project-specific tasks at hand. Written and verbal information is provided to the responsible employees so that continuity of service and understanding of the clients needs and protocol requirements are in place. Laboratory Managers also communicate with clients to ensure that the scheduling, workflow, and data quality meet the project objectives.

With the approval of the Director, Managers may also afford clients (or their approved representatives) opportunities to clarify their requests or work plans and will monitor laboratory performance in relation to the work performed.

4.2.3 Laboratory Analysts

Laboratory analysts are responsible for the generation of data by analyzing samples according to written SOPs or project protocols. Analysts report to their respective Department Manager. They are also responsible for ensuring that documentation related to specific samples is complete and accurate. Analysts provide their Department Manager with immediate notification of problems within the laboratory that could potentially affect the quality of the data. While Managers and the Director have the final authority to accept or reject data (based on compliance with well-defined QC acceptance criteria), laboratory analysts often conduct a preliminary review of data to assess acceptability of the data. Acceptance of data, which falls outside of QC criteria or is questionable in nature, must be reviewed and approved by the Department Manager or the Director. Analysts also provide support in organism culture and handling, water and media preparations, glassware cleaning, and many other tasks associated with microbiological, toxicological, or ecological tests and investigations.

It is expected that employees will generate data in compliance with this QAPP and the Employee Manual.

4.3 TRAINING

The QAPP, The Employee Manual, and our comprehensive Standard Operating Procedure (SOP) program provide the foundation for training new or current employees. The QAPP and the Employee Manual are required documented reading for new employees. The new employee must also sign an “agree-to follow” statement as part of the documentation record. As the employee gains experience and begins to become responsible for implementation of specific methodologies or protocols, the associated SOP must be read and understood and documented before an analyst becomes qualified for that procedure. The Laboratory Manager or a qualified analyst will also provide a verbal description and demonstrate the procedure to the trainee.

Records of initial training for analyses under the National Environmental Laboratory Accreditation Program (NELAP) will be recorded on the “Demonstration of Capability” form (Appendix E). This form, along with supporting documentation should be stored in the personnel files for the trainee analyst.

4.3.1 Orientation

Personnel training procedures begin with an orientation program designed to familiarize the new associate with safety and chemical hygiene issues, the importance of quality assurance/quality control in the analytical laboratory, and company policies and benefits. New personnel are also educated in the ethical and legal responsibilities of the work and recording or reporting data, as outlined in the Employee Manual. This includes potential punishments or penalties for improper, unethical, or illegal actions (Employee Manual Section A. 8.). The new employee must also sign an “agree-to follow” statement as part of the documentation record for the Employee Manual and this QAPP.

Data integrity is the foundation of our work at Aquatec Biological Sciences, Inc. Our initial and routine training includes discussion of the importance of data integrity and how we achieve this objective. Key components of our data integrity system include:

- Record observations in real time;
- Correct recording errors in a manner that doesn’t obliterate original records;
- Prevent improper data manipulations;
- Maintain confidentiality regarding the specifics of our work (unless authorized to release information by our clients);
- Maintain an environment of honesty and full disclosure in analytical reporting;
- Communicate to employees that breach of ethical behavior could result in investigative actions, including possible termination or prosecution.

4.3.2 Routine Training

The level of training necessary to perform analytical tasks is derived from academic

background, past experience, technical courses, and on-the-job training with specific methods or instrumentation. The responsibility for formal academic training lies foremost with the individual and educational background checks are fundamental to hiring. The responsibility for the additional specialized skills obtained through in-house training or external workshops is a shared obligation of the individual, their supervisor, and the laboratory.

An individual's academic and professional experience is kept on file including an initial statement of qualifications or resume and any additional documentation concerning subsequent training. Copies of certificates of completion, transcripts, diplomas, or other documentation will be included in the files as appropriate.

An employee's training file must include documentation that the employee has read, understands, and is using the latest version of our Quality Systems documentation (QAPP and the Employee Manual). The Employee Manual outlines the ethical and legal responsibilities, including the potential punishments and penalties for violations. In order to ensure that the policies and objectives of the QAPP are communicated to all new personnel, employees are required to read the QAPP during the training process. The employee must sign that the current version of the Quality Systems documents have been read, understood, and agreement to follow. Training on specific instrumentation or laboratory procedures is SOP-driven, with signature documentation of reading and understanding and "agreement to follow" for SOPs. Documentation of training must be included with the employee's training records. Training records are available for inspection by appropriate regulatory or certifying authorities.

Trainees are under the supervision of experienced analysts who are responsible for showing them the analytical procedures including applicable QA/QC measures. A new analyst will not be permitted to fully implement an analysis until their supervisor is confident that the analytical and QA/QC procedures can be carried out correctly and method proficiency is documented (Demonstration of Capability (Appendix E)).

4.3.3 Documentation of On-Going Proficiency

Aquatec analysts must remain proficient in the tests that they perform. Quality Systems documents and SOPs are periodically revised as part of the annual Quality Systems review and analysts must complete documented reading (including statement of understanding and "will follow" statement) of the latest versions of these documents as they are published. SOPs are revised to reflect advances or changes in regulatory-based protocols.

Analysts will be considered up to date if their training file contains documentation that they have read, understood, and agreed to perform the most recent version of the test method (as outlined in approved SOPs). In addition, documentation of continued proficiency must be completed at least annually by one of the following:

1) Documented ability to meet negative control acceptance criteria.

In the Toxicology Lab: by demonstrating the ability to meet method acceptance criteria in the laboratory controls.

In the Microbiology Lab: by demonstrating the ability to meet method acceptance criteria in the laboratory controls.

2) Ability to conduct and submit acceptable DMR or PE studies (blind samples).

In the Toxicology Lab: by completing DMR WET studies on an annual basis with results within the DMR acceptable range. The current provider is Environmental Resource Associates (ERA).

In the Microbiology Lab: by completing PE studies on a bi-annual basis, with results within the acceptable range of the study. The current provider is Environmental Resource Associates (ERA).

3) Documented ability to meet positive control criteria:

In the Toxicology Lab: by performing standard reference toxicant tests with results within acceptable control chart ranges

In the Microbiology Lab: by performing positive controls and having positive controls meet performance based criteria.

These records must be filed in the analysts training records.

4.3.4 Work Cells

Work Cells are defined as a group of analysts working on parts or cooperatively on all of a laboratory method and generating data for that method (e.g., a toxicity test may require different analysts to perform different tasks as part of the overall method or may perform the same tasks for a method but on different times or days). Analysts comprising Work Cells must, as a unit, meet the requirements outlined in Section 4.3.3. Each member of a work cell must meet the requirements of 4.3.3, such that the individual can participate in any aspect of work assigned to the work cell. Some participants in a work cell may be qualified for limited portions of the overall assignment. These individuals must have documented training for their specific assignments.

4.3.5 Safety Training

Aquatec has a fundamental responsibility to provide facilities, equipment, maintenance, and an organized program to make necessary improvements to ensure a safe working environment. Unless employees fulfill their responsibilities for laboratory safety, the safety-related features of the facility and established safety programs will be ineffective.

The laboratory is equipped with many structural safety features. Each associate must be familiar with the location, use, and capabilities of general and specialized safety features associated with their workplace. To protect employees from potential workplace hazards, Aquatec provides and requires the use of certain items of protective equipment. These may include safety glasses, protective clothing, gloves, respirators, etc., as needed for the tasks at hand. Repeated violations of published safety practices (Employee Manual) may result in punitive action ranging from required safety training to employment termination.

5.0 PROCEDURES FOR HANDLING SAMPLES

Sample integrity is the foundation upon which meaningful biological results rely. For field sampling, an approved Sampling Plan should be developed. The Sampling Plan should define the data quality objectives relevant to the assessment goals specific to the sampling site. The integrity of samples must be maintained through the use of appropriate sample preservation techniques as specified in project-specific protocols or work plans. Samples should be submitted to the laboratory under standard Chain-of-Custody (CoC) procedures. Samples submitted to the laboratory are logged in as soon as possible. Any sample that is suspected of being contaminated, improperly stored or preserved, or improperly prepared, is documented on the Chain-of-Custody form and included as a “qualifier” in the report. Instances where sample condition varies widely from normal are reported to the Department Manager and/or client immediately. Guidance for storage and final disposal of samples is located in Appendix B of this QAPP.

5.1 CHAIN-OF-CUSTODY PROCEDURES

The critical nature of Chain-of-Custody procedures cannot be overemphasized. These procedures generate a historical record of the samples' custody from the time of acquisition to time of completion of the analysis. In most cases, samples are stored beyond the analysis time and then discarded following project-specific guidelines or in-house protocols for sample disposal (Appendix B to this QAPP).

The Chain-of-Custody procedures employed at Aquatec are implemented through our Laboratory Management System (LMS) or with client-generated Chain of Custodies. An example Chain-of-Custody form is presented in Figure 4 (Appendix E). The following procedures have been established to ensure samples are secure and properly stored.

- Access to the laboratory is through a reception area with all other access doors locked. Visitors are escorted during their time in the laboratory.
- If requested, refrigerators, freezers, and other sample storage areas can be locked. Samples remain in sample storage until removal for sample preparation or analysis.

- If requested, transfers of samples into and out of the storage area(s) can be documented on an Internal CoC record (Figure 5, Appendix E). The assigned sample custodian, or his designate, will control the internal custody of samples.
- After a sample has been removed from storage for analysis, the analyst is responsible for returning the sample to the storage area before the end of their working day.

5.2 SAMPLE RECEIPT AND SAMPLE ACCEPTANCE POLICY

SOP TOX1-017 is a companion document containing additional information regarding sample acceptance. Authorized Aquatec personnel receive samples at the laboratory. Samples typically arrive in coolers with thermal preservation (iced). The cooler is opened and a thermometer is placed inside the cooler to measure the ambient temperature. The ambient temperature is recorded on the CoC form. Some samples do not require thermal preservation (e.g., macroinvertebrate samples are typically preserved with formalin or ethyl alcohol, samples for metals analysis (subcontracted) or total ammonia are preserved with acid). Samples are removed from the cooler and sample labels are compared with the sample descriptions on the CoC form. The analyst must provide initials on the login form for identification. The samples are assigned a unique laboratory identification number (sequential six-digit number) and logged in for the requested parameter(s). The sample number is maintained electronically (Access data base) and on paper in the form of work sheets. A sample number label is also physically affixed to the sample container(s). The sample number is the key mechanism for tracking any sample throughout the laboratory.

We provide information to our clients regarding the proper sample preservation and storage conditions during shipping. While most samples arrive within the specified conditions, occasionally samples arrive outside the target boundaries (e.g., samples that are recently collected may not have cooled to 6°C when they are delivered to us.). Discrepancies in sample condition must be documented on the CoC form and the Laboratory Manager should be notified so that possible corrective action can be initiated. The resolution of discrepancies will be noted either on the CoC record, the log-in sheet, or a document stored in the project file. If the cooler temperature exceeds 7°C, the Laboratory Manager or the Director is notified. The Laboratory Manager or the Director will make a decision as to whether the client should be notified to warn of the discrepancy. Communication with clients regarding potential breach in sample integrity (e.g., too warm, broken sample containers), can take the form of documented phone calls, Fax, or e-mail, and client's authorization to proceed with the analysis. Many biological analyses are time-sensitive (i.e., tests must be started within holding times). Therefore, it may be more important to go ahead with the analysis and report the result as "qualified" rather than stall the analysis for unresolved sample condition issues.

If the sample does not meet sample receipt acceptance criteria, correspondence or records of final disposition of rejected samples should be documented and stored in the project file. Reports for analyses that proceed with samples not meeting acceptance criteria must be qualified by recording the condition of the samples on the chain-of-custody form or associated paperwork. This documentation should be included in the report package, with appropriate qualifiers included in the report.

Aquatec utilizes the LMS to track samples and analytical data. These data include:

- Sample number (unique to each sample)
- Date received
- Initials of analyst logging in the samples
- Date analytical results due
- Sample descriptions
- Additional comments
- Client's name
- Client's address
- Client's job number (if available)
- Notation of any special handling instructions or priority assignments
- Billing information - purchase order numbers (if available)
- Analyses requested

5.2.1 Biological Test Request

The information listed above becomes embedded in the Biological Test Request (BTR). The BTR is generated in the Laboratory Management System for each sample or series of samples. The BTR contains all of the specific information relative to a particular sample or group of samples. BTR worksheets are printed from LMS and are also stored electronically. The BTR worksheets accompany the sample during the analysis and are maintained in the project file along with the data generated during the analysis and the final report. The LMS allows us to track samples from log-in through analysis, reporting, and final invoicing.

The method-specific analytical worksheets for a BTR are delivered to the analyst(s) along with associated bench-sheets. When the laboratory section is ready to analyze a sample, the analyst retrieves the sample. If Internal Chain-of-Custody (ICoC) is required (not required unless specified by the client) for a specific sample or group of samples, the analyst is required to sign and record the date and time on the ICoC form when removing the sample(s) from the storage area. When the analysis is complete, the analyst returns the sample to the storage area and relinquishes custody by again signing the ICoC form (if required). Samples are stored in the refrigerator or freezer (depending on project requirements) until disposal following completion of the analyses (See Section 9.0 for guidelines regarding sample disposal).

5.2.2 Sample Delivery Groups

Sample Delivery Groups (SDGs) link a series of BTRs together for reporting purposes. Often a series of samples, delivered on different days, are required to complete an analysis (e.g., for chronic whole effluent toxicity tests where renewal samples are required) or to meet the experimental design requirements (e.g., for sediment toxicity tests where a series of samples are tested or reported concurrently). The BTRs are assigned to a SDG, defined as the first BTR number assigned for any group of samples being linked in this way.

5.2.3 Subcontracted Analyses

Some samples received at Aquatec require analyses that are not routinely performed here (e.g., chemical analyses). These analyses may be subcontracted to qualified laboratories for the specific analysis. When appropriate the sub-contract laboratory may be assessed by the laboratory either through an on-site visit or by the submission of sufficient documentation to determine the sub-contractor's capabilities and qualifications. As a minimum, certification is required from NELAC and/or the State where the samples originated. Other required documentation may include laboratory QAPP, SOP's, recent PE sample results, or other relevant certifications. Samples being analyzed for NELAC-accredited parameters should be sent to a laboratory that is NELAC-accredited for the required parameters.

Samples for subcontracted analyses are shipped to laboratories under CoC protocols. Reports received from subcontracted laboratories are reviewed at Aquatec and incorporated into the final report sent to the client.

5.3 CALIBRATION

5.3.1 Certification and Calibration of Reference Weights and Thermometers

5.3.1.1 NIST Traceable Thermometers

Aquatec's ERTCO mercury thermometers, that are used as a reference standard for calibration of laboratory thermometers or probes, must be calibrated to a NIST Traceable standard every two years by an accredited external source such as:

Barnstead International
2555 Kerper Boulevard
Dubuque, IA 52001
Phone: (563) 556-2241

The report of certification must be filed in Aquatec's Balance and Temperature Monitoring Logbook. At each certification event, a label will be applied to the certified thermometer indicating the date of certification and the date of expiration. Reference thermometers not re-certified should be labeled as "expired" and not be used.

5.3.1.2 Reference Weights

Troemener Reference weights used for balance calibration checks must be calibrated to NIST traceable standards annually by an accredited external source such as:

Mettler Toledo, Inc.

1900 Polaris Parkway
Columbus, OH 43240

Contact: Richard Harris (800) 786-0034 ext. 7122

The report of certification must be filed in Aquatec's Balance and Temperature Monitoring Logbook. At each certification event, a label will be applied to the reference weight storage box indicating the date of certification and the date of expiration.

5.3.2 Other Calibration Standards

Analytical standards or reference materials used for calibration include commercial reagents, stock solutions, working solutions, calibration standards, and instrument check standards.

Materials used for preparation of other laboratory standard solutions are purchased from suppliers capable of providing certificates of analysis or purity data. Where applicable, certified reference materials or client-supplied certified analytical reference materials are used. When reference materials, standards, and commercial reagents are received, they are logged into a permanent logbook and are marked with the receiving date, lot number and expiration date. Expiration dates (if available) are checked before any reference material is used within the laboratory. Reference materials, standards, and commercial reagents are also marked with the date of first opening.

Stock solutions are prepared by dissolving known amounts of reference materials, standards, or commercial reagents in a suitable solvent (usually deionized water). Alternatively, stock solutions may be purchased from vendors capable of supplying appropriately certified solutions. Whether prepared in the laboratory or purchased from a suitable source, the following items must be recorded on the bottle containing the stock solution: Contents; Concentration(s); Date prepared, Analyst, and Expiration Date.

5.3.3 Instrument Calibration

Calibration of instrumentation is required to ensure that the analytical system is operating correctly and functioning at the proper sensitivity to meet established reporting limits. Each instrument is calibrated with standard solutions appropriate to the type of instrument and the linear range established for the analytical method.

Instrument-specific SOP's discuss in detail how each instrument is calibrated, including frequency for calibration and re-calibration, and the source of the calibration materials.

A variety of instruments and wet chemical techniques are available to support microbiological, toxicological, and ecological tests or investigations. Calibration and standardization procedures vary depending on the instrumentation and analytical methodology required for a specific analysis. The calibration is checked on an ongoing basis to ensure that the system remains within specifications. If the ongoing calibration check does not meet established criteria, analysis is halted and corrective action taken. The procedures include assessment of instrument performance, recalibration, reanalysis of check standards, and possibly reanalysis of samples.

Calibration of dispensing pipettors is performed quarterly.

5.4 SAMPLE ANALYSES

Detailed descriptions of procedures for the analyses of samples are maintained in laboratory method SOP's. Appendix C of this QAPP presents a summary of the methods employed by Aquatec.

In general, departures from documented policies and procedures or from standard specifications are not permitted. Certain sample matrices or client requests may require method development. In cases where a procedure is modified from standard protocol, the client will be informed of the changes to that protocol. The changes will be documented in a project-specific SOP and/or described in a narrative or qualifier, which accompany the analytical results.

5.5 ANALYTICAL METHODS

The following documents describe the majority of analytical methods performed at Aquatec:

EPA 600/4-90/027F, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 1993.

EPA-821-R-02-012, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Edition October 2002.

EPA 600/4-91/002, *Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms*. 1994.

EPA-821-R-02-013, *Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms*. 4th Edition, October 2002.

EPA 600/4-91/003, *Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Marine And Estuarine Organisms*. 1994.

Aquatec Biological Sciences, Inc.

EPA-821-R-02-014, *Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Marine And Estuarine Organisms*, 3rd Edition, October, 2002.

EPA 600/R-99/064, *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Second Edition, 2000.

EPA 600/R-94/025, *Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods*, 1994.

EPA 600/4-90/030/, *Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters*, 1990.

EPA 841/B-99/002, *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish*, Second Edition, 1999.

EPA 600/4-79/020, *Methods for Chemical Analysis of Water and Wastes*, 1983.

EPA 600/R-93/100, *Methods for the Determination of Inorganic Substances in Environmental Samples*, August 1993.

EPA SW-846, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*, 3rd Edition, with Update I, Update II, and Update IIA, September 1994.

EPA / USA CoE NED, *Regional Implementation Manual for the Evaluation of Dredged Material Proposed for Disposal in New England Waters*, April 2004

EPA 823-B-98-004 *Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Testing manual, Inland Testing Manual*, 1998

EPA / A CoE, *Evaluation of Dredged Material Proposed for Ocean Disposal (Testing Manual)*, 1991

EPA/600/6-91/003; *Methods for Aquatic Toxicity Identification Evaluations, Phase 1 Toxicity Characterization Procedures*, 1991

EPA/600/6-91/005F; *Toxicity Identification Evaluation, Characterization of Chronically Toxic Effluents, Phase 1*, 1992

EPA/600/R-92/080; *Methods for Aquatic Toxicity Identification Evaluations, Phase 2 Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity*, 1993

EPA/600/R-92/081; *Methods for Aquatic Toxicity Identification Evaluations, Phase 3 Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity*, 1993

EPA/600/R-96/054; *Marine Toxicity Identification Evaluation (TIE), Phase 1 Guidance Document*, 1996

APHA/AWWA/WPCF, *Standard Methods for the Examination of Water and Wastewater*, 19th Edition.

ASTM, *American Society for Testing & Materials; Annual Book of Standards*, 2000. Volume 11.05 Biological Effects and Environmental Fate; Biotechnology; Pesticides.

ASTM, *Standards on Materials and Environmental Microbiology*, 2nd edition. 1993.

NOAA (National Oceanic and Atmospheric Administration), *National Status and Trends Program*, Volume I-IV, 1984-1994.

Plumb, Russell, USEPA Corps of Engineers, *Procedures for Handling and Chemical Analysis of Sediment and Water Samples*, May 1991.

FDA, *Bacteriological Analytical Manual (BAM)*, 8th edition. 1995.

AOAC, *Official Methods of Analysis*, 14th edition. 1984.

5.6 DATA REDUCTION

Each Department provides thorough data review prior to reporting results to the client. In general an analyst will process data in one of the following ways:

- Manual computation of results with manual reporting
- Computer computation of results with manual reporting
- Computer computation and reporting of results

If the analyst manually processes the data, all steps in the computation are provided for review including the source of the input parameters such as response factors, dilution factors, and calibration constants. All calculations of manually processed data are checked during secondary review.

For data that is processed using a computer and then entered into the LMS by an analyst or data entry personnel, a hard copy of the computer-generated results is used along with any other preparation or dilution information as may be needed. The hard copy results are used for data validation and secondary review. This process is

iterative, to that point that no known entry or computational errors are detected.

If computer-processed data is directly acquired from the instrumentation, hard copies of the actual data are made and the analyst verifies that the following are correct before releasing instrumental data to the reporting system:

- sample numbers
- calibration constants/ response factors
- output parameters such as units and identifiers
- dilution and preparation factors

The hard copy of the results is used for data validation and review. After initial demonstration of proficiency of computerized programs, computer calculations are periodically spot checked for consistency and accuracy. Commercially available statistical programs utilized in data analysis are checked annually using a known data set.

5.7 DATA REVIEW

The analyst is responsible for preliminary review of data generated from sample analysis. If the instrument calibration and/or quality control samples are within specified tolerances, then the data are presented to data reviewers for secondary review. If instrument calibration or the quality control samples exceed specified tolerances, then affected sample results are evaluated and the samples may be submitted for re-analysis, a re-sampling may be requested of the client, or the results may be submitted as “qualified”. If discrepancies or deficiencies exist in the analytical results, then an appropriate corrective action may be required.

Secondary review is conducted by Laboratory Managers or designated analysts who review the bench-sheet data determine whether analytical results are acceptable. Also, calibrations, manual calculations, and transcriptions are checked for accuracy. Quality control sample results are evaluated against the specified criteria. If instrument calibration and quality control samples are acceptable, then the data are presented for final review.

The final level of review is conducted by the Director / Quality Assurance Officer or Laboratory Managers, to confirm that there is reasonable agreement in the findings from a technical perspective.

5.8 DATA REPORTING

After analytical data have been reviewed, the final report can be assembled for

submission to the client. The completed report and supporting data package are reviewed to verify that the final results are presented in a clear and concise manner.

Aquatec reports data associated with biological analyses. Typically these data do not fit within the strict definitions of Levels 1-5 common to analytical chemistry laboratories. Our data are reported within the general provisions of Level 2 (e.g., microbiology and ecology reports) and Level 4 (e.g., microbiology, ecology, and toxicity reports) where associated laboratory bench sheets and Chain-of-Custody forms are included as appendices to the reports. Field measurements generally meet Level I data requirements.

The various data defined data levels(US EPA) are as follows:

Level I data consists of measurements taken during field analysis with the report consisting of results only.

Level II reporting consists of an analytical report with results only. Internal quality control results are retained on file at the laboratory.

Level III reporting consists of an analytical report with internal quality control results reported; these include laboratory control standards, surrogate spike recoveries, and method blank results.

Level IV data refers to data submitted in CLP-like format. Level IV is defined by the submission of QA/QC supporting material including the raw laboratory data similar to that provided with CLP Statements of Work (SOW). Submission of data in this format results in a package that can be independently validated. Level IV reporting includes narrative, analytical results, supportive documentation including raw data and preparation sheets, and documentation related to Chain-of-Custody. Once the document is assembled, the report sections are distinguished with title pages. Copy(s) of the documentation are sent to the client, and the original document is retained at Aquatec in storage for a minimum of five (5) years.

Level V data has unique requirements in either compound identification, quantification, detection limits, cleanup or QA/QC requirements. Level V analytical procedures are generally defined as Special Analytical Services. The procedures and QA/QC are specified through these requests. The QA/QC for Level V data usually requires frequent standardization, spikes, duplicates, blanks, and strict compound identification criteria. This level of reporting is not typical of the biological analyses performed at Aquatec.

Analytical results that are transmitted by phone, facsimile, or electronically (e-mail) should be done only at the request of a known and appropriate representative of the client. For microbiology results, a pre-arranged and paid agreement is requested of the client and this is documented on the microbiology analysis Chain-of-Custody form. For other analytical results submitted by facsimile or e-mail, the facsimile form should

contain a confidentiality statement. Occasionally, known clients wish to be apprised regarding the progress or outcome of analyses. In these cases, the analyst or project manager should be reasonably sure that the correct client contact is being informed and unless the data or the analysis have gone through final review, the information should be presented as “preliminary”.

The standard report must contain the following elements:

- Title;
- Aquatec Biological Sciences, Inc. (for analyses conducted here);
- Identification of laboratories used for any subcontracted analyses;
- Unique identification of the test report (SDG number, sample numbers);
- Project name and name and address of the client;
- Test method identification;
- Pagination for multiple page reports.

The body of toxicity reports contains tabulated data in the form of a Summary Report; Detail Report (e.g., replicate data), and a Quality Control section. The Quality Control section incorporates calculated values of data quality indicators (i.e., control treatment performance specific to the test method) and a discussion of any qualifiers for the reported results. See also Section 6.5.2.6. Reports for sediment evaluations include a Quality Control Summary Table with data quality indicators and test acceptance criteria clearly identified.

6.0 QUALITY CONTROL

6.1 LABORATORY WATER

The quality of our base water and reconstituted waters is of primary importance to our functioning as an aquatic laboratory. Aquatec has a water-quality monitoring program in place to insure that high quality standards are met. Details of the monitoring program are outlined in SOP TOX1-018. As a minimum, our base water (deionized water used for final rinses of cleaned glassware and for preparation of reconstituted waters) will be monitored annually for organics (pesticides and PCBs) and metals as specified in EPA-600-4-91-002 and other EPA documents. An annual water suitability test (SM 9020B.3.C) is also performed. Our base water is monitored monthly for total residual chlorine (TRC), pH, and total Kjeldahl nitrogen (TKN), total organic carbon (TOC) and total bacteria. Our reconstituted waters, used for dilution water and as laboratory controls in toxicity tests are monitored for TRC, total ammonia, total and suspended solids, total organic carbon, and metals at least quarterly. Samples associated with this monitoring program are submitted to a laboratory certified for the requested analysis. Results of the analyses (analytical reports) are reviewed and maintained on file for the current year and archived for a minimum of five years.

The water purification system consists of particle removal pre-treatment (2), carbon bed

(1), reverse osmosis (R / O) membrane (1), deionizing resin beds (2), 300 gallon storage tank with a continuously circulation delivery loop powered by a Dayton 6K578C pump. Higher levels of water purification are attained by passing R/O deionized water through the NanoPure system, resulting in 18.3 megohm-cm quality water.

The following maintenance schedule should be followed for this system:

- Daily: NanoPure quality check: allow to run ~1 min. and record meter reading in the Toxicology Laboratory Daily Calibration Log;
- Daily: check the warning light on the deionizing resin beds. If the light goes to red, notify the Laboratory Manager or the Director. Replacement of one or both resin beds and the carbon bed may be required.
- Weekly: record pressure and performance parameters on the weekly check list. If anything appears to be abnormal, notify the Laboratory Manager or the Director.
- Every three months: Pretreatment Filter 1 (DGD-2501-20 dual density gradient available locally from Culligan) and Filter 2 (Fisher 09034162, 0.5 micron);
- Every six months: NanoPure D0749 (Fisher 0903449) 0.2 micron filter;
- Every 18 months: NanoPure D0835 (1 pre-treatment); D0809 UltraPure (2);
- As needed (based on warning light), typically every 6 months: carbon bed and deionizing resin beds (Culligan Water Conditioning, 865-0000).

6.2 FOOD STOCKS

Organisms used in toxicity tests are expected to be healthy and nutritionally satisfied before and/or during testing. Food stocks used for organisms in toxicity tests must be of a quality that will support survival, growth, or reproduction levels that will meet or exceed minimum test-acceptability requirements established by the test protocol. *Artemia* (brine shrimp) eggs are purchased from a commercial supplier. Each new batch or lot of *Artemia* will be tested for successful hatchability and support of survival and growth in fathead minnows (based on control performance). Survival and growth in laboratory water must meet or exceed the EPA criteria of 80 percent and 0.25 mg (average dry weight), respectively.

Food stocks used for culturing the water flea, *Ceriodaphnia dubia*, include the alga, *Selenastrum capricornutum*, and Yeast-Cerophyl-Trout Chow (YCT) obtained from a commercial supplier such as Aquatic Biosystems of Fort Collins Colorado. *Selenastrum* is stored refrigerated with a shelf life limited to two weeks. YCT is stored frozen, with a shelf life of 1 year. These stocks are used to maintain the brood boards leading up to toxicity test use. The brood board organisms are tracked for survival and reproduction daily during the week preceding the use of their offspring for testing. It is during this time that the quality of the food stocks is verified. If a depression in survival or reproduction appears to be linked to a particular batch of food, a new batch is acquired as soon as possible.

In-house cultures of the sea urchin, *Arbacia punctulata*, are fed fresh Romaine lettuce acquired from local grocery stores. This food source has been adequate to support gravid adult sea urchins for years at Aquatec.

6.3 DATA QUALITY OBJECTIVES

The data quality objectives discussed below ensure that data will be gathered and presented in accordance with procedures appropriate for their intended use, and that the data will be of known and documented quality able to withstand scientific and legal scrutiny. The quality of the measurement data can be defined in terms of precision, accuracy, representativeness, completeness, comparability, and traceability.

6.3.1 Precision

Precision is the determination of the reproducibility of measurements under a given set of conditions, or a quantitative measure of the variability of a group of measurements compared to their average value. Precision is typically measured by analyzing field duplicates and laboratory duplicates (sample duplicate, matrix spike duplicate, and/or laboratory duplicate). Precision is most frequently expressed as standard deviation, percent relative standard deviation, coefficient of variation, or relative percent difference. Precision goals are specified in most methods. Whole effluent testing protocols typically require within-test replication, resulting in a measure of precision inherent to the test and reflected in the statistical analysis. Precision measures may not be applicable to some biological analyses.

6.3.2 Accuracy

Accuracy is the measure of agreement between an analytical result and its "true" or accepted value. Deviations from a standard value may result from a change in the measurement system. Potential sources of deviations include (but are not limited to) the sampling process, sample preservation, sample handling, matrix effects, and sample analysis. Analytical laboratory accuracy is determined by comparing results from the analysis of matrix spikes, surrogates, or check standards to their known values. Accuracy results are generally expressed as percent recovery and accuracy goals may be specified in methods. Accuracy measures may not be applicable to some biological analyses.

For microbiology and toxicity analyses measures of accuracy, and an assessment of our performance with unknown samples, are generated through participation in annual or bi-annual US EPA DMR studies. Our performance in these studies is made available to appropriate NELAC personnel for accreditation purposes and to regulatory agencies or clients requesting the results.

6.3.3 Representativeness

Representativeness of a sample is determined by the sampling personnel in collecting a representative sample and the laboratory using a representative portion of the sample for analysis. The laboratory makes every effort to ensure a representative aliquot is removed from the sample container. Homogenization of the sample (e.g., for sediment samples) may be carried out in the laboratory when appropriate.

6.3.4 Completeness

Completeness is defined as the percentage of measurements that are judged to be valid measurements. Factors negatively affecting completeness include the following; sample leakage or breakage (in transit or during handling), violating specified holding times, loss of sample during laboratory analysis through accident or improper handling, improper documentation such that traceability is compromised, or rejection of sample results due to failure to conform to QC criteria specifications. A completeness objective of at least 90% of the data is the goal established for most projects. Aquatec strives for a completeness goal of 100%.

6.3.5 Comparability

Comparability of results between current and past sampling events, and between analytical sequences of a method is achieved through Quality Assurance Project Plans (QAPP), controlled SOP's, and experienced well-trained analysts.

6.3.6 Traceability

Traceability is the extent to which reported supporting documentation can substantiate analytical results. Trace-ability documentation exists in two essential forms: those that link the quantification process to authoritative standards, and those that explicitly describe the history of each sample from collection to analysis and disposal. Aquatec strives for a traceability goal of 100%.

6.4 QUALITY CONTROL MEASURES

The quality control program implemented at Aquatec includes the analysis of check standards and appropriate control treatments. Analytical sample series include these controls, specific to the analysis. The controls used in an analysis should be completely representative of the procedure, including sample preparation and sample analysis.

The following describes procedures used to monitor method performance and support data validation. Control treatments analyzed in conjunction with samples are essential to the evaluation of the data quality. The following quality controls may be incorporated into specific analytical procedures, biological tests, or investigations.

More specific Quality Control measures for the Microbiology Laboratory are detailed in the QASOP-2006, Revision 6.

6.4.1 Method Blanks

Method blanks are prepared and analyzed with an analytical batch of twenty or fewer samples to identify possible sources of contamination within the analytical process. Method blanks are treated as samples (ie they must go through each stage of the analytical process), including glassware, reagents, instrumentation, exposure, and any other source of possible contamination that may affect sample results. For some biological analyses, the method blank may be represented by the negative control (e.g., whole effluent toxicity tests). For other biological analyses such as bioaccumulation tests, a representative sub-sample of the test organisms may be submitted to the analytical laboratory to assess “background conditions” of the organisms. If applicable, the control limits and corrective actions for method blanks are defined in the method SOP's.

One negative control sample and one blank are run with each daily set of coliform and *E.coli* samples to assess the analytical process

6.4.2 Laboratory Control Samples (Negative Controls)

Laboratory Control Samples (LCS) are prepared and analyzed with each batch of samples (samples tested concurrently) or with individual samples. Aqueous and/or solid (sediment or soil) laboratory control samples are analyzed using sample preparation, environmental conditions, and analytical methods representative of the methods employed for the samples received. LCS samples, or negative controls, are typically incorporated within each test method. The control limits and corrective actions for LCS's are defined in the method SOP's and relevant guidance documents.

For *E. coli* analyses the negative control organism is *Enterobacter aerogenes* and for total coliform analyses the negative control organism is *Pseudomonas aeruginosa*. These negative controls are analyzed daily or with each set of 20 samples for the corresponding method.

6.4.3 Laboratory Control Samples (Positive Controls)

Positive control samples for the microbiology laboratory are prepared to known densities of the organism (e.g., *E. coli*), which should return an expected and measurable growth pattern each time it is prepared. Positive controls are performed concurrently with the analysis of the samples received.

Positive controls for the toxicity laboratory are represented by the Standard Reference

Toxicant (SRT) tests that are routinely performed with test organisms. In this quality control program, a known toxicant is prepared, and a reference toxicant test is initiated with a representative sub-sample of the test organisms. A species should respond in a consistent way to a presented toxicant when tested repeatedly over time and under similar conditions (age and environmental).

Toxicity values (LC50 or IC25) are generated from SRTs and they are plotted on a control chart. The boundaries of acceptability are \pm two standard deviations on the plotted control chart. Statistically, one in twenty test-values, may fall outside the control chart boundaries due to chance alone. If a SRT value falls outside the expected range, the data associated with that test should be reviewed to determine whether laboratory error contributed to the deviation. If a laboratory error is identified as a contributing factor, the SRT test should be repeated. If the SRT test is outside of the boundaries and no laboratory errors have been identified, variability in organism sensitivity or health may be a factor and also, could be reflected in responses observed in associated effluent or sediment toxicity tests. These test data should be interpreted with caution and may need to be qualified or repeated. SRT tests are performed quarterly, monthly, or concurrently with client-based samples. More specific requirements for the SRT program are outlined in SOP TOX4-001 and the individual SOPs describing SRT procedures for each species.

6.4.4 Replicate Analyses

Replicate analyses (e.g., field or laboratory duplicates) are often received with groups of samples requested for analyses. These client-provided duplicates are typically treated as an independent sample and are included in the concurrent testing group, when requested by the client. Toxicity testing protocols include laboratory replicates (of each sample or treatment level) inherent to the test method, as outlined in the guidance documents and Aquatec SOPs for the individual tests.

6.4.5 Calibration Check Standards

Microbiological analyses for total coliform are conducted monthly as replicates of a positive control for precision evaluation. These analyses include running a known control in duplicate. Also, on a monthly basis, a countable positive total coliform or *E. coli* is counted independently by two analysts for agreement.

In the Toxicity Laboratory, calibration check standards are used daily to calibrate and evaluate the performance of water quality instrumentation.

6.4.6 Statistical Quality Control

Statistical control charting is performed for methods to monitor the precision and/or

accuracy of the laboratory's performance using the particular methodology. Software programs are used for management and compilation of laboratory control charts. Control limits are calculated in terms of multiple standard deviations from a mean or other reference point. Warning limits are set at ± 1 standard deviations and control limits are set at ± 2 standard deviations. Initial limits are established after a minimum of five to twenty data points depending on the analysis. Once established, the control limits are updated whenever more data points are generated, and the control chart is maintained based upon the most recent 20 values.

Statistical control charts are plotted for standard reference toxicant tests on aquatic species tested. These control limits, once calculated, are used to monitor the quality of test organisms and laboratory techniques. When analysis of a standard reference toxicant is completed the quality control data is reviewed and evaluated against these limits (most recent 20 values).

Commercially available statistical programs utilized in data analysis are checked annually using a known data set.

6.4.7 Equipment Quality Control

6.4.7.1 Volumetric Calibration Checks

When pre-sterilized, disposable pipettes are used, packs are opened and resealed between major uses, to maintain sterility. Pipettes are checked quarterly for volume accuracy. Each new batch of pre-sterilized, disposable pipettes needs to be checked for volume accuracy, the same as pipettors.

All pipettes need to be checked using a volume measurement normally used, and be within 5% accuracy of that volume. Tare a small cup or weigh-pan on the balance, pipette the volume of de-ionized water into the cup. The volume should equal the weight, grams = mL $\pm 5\%$. If a lot of pipettes does not meet this criterium, it will be rejected, and a new lot ordered. The results are recorded in the Pipette calibration section of the Calibration Log Book.

6.4.7.2 Autoclave Operational Quality Control

The autoclave operation is checked monthly using "BT Sure" ampules. If a failure of BT Sure results during the monthly testing, it must be re-tested after a complete check of autoclave operation has been conducted. Two consecutive failures requires maintenance from a specialist and notification of the director.

The autoclave timer mechanism is checked quarterly for accuracy, and any corrections are recorded and applied.

A safety-valve check is also conducted quarterly while the autoclave is in operation.

Records of autoclave operations are maintained for each cycle, including: date, contents, maximum temperature reached, pressure, time of sterilization mode, total run time, and analyst's initials.

6.5 QUALITY CONTROL STANDARDS

6.5.1 Microbiology

More specific Quality Control measures for the Microbiology Laboratory are detailed in the QASOP-2006, Revision 6.

6.5.1.1 Sterility Checks

All equipment and supplies used in the microbiological analyses are routinely checked for sterility. Laboratory de-ionized water is monitored monthly for bacterial densities and required water quality characteristics. If any of the parameters are out of the specified tolerances corrective action is taken.

The laboratory water is tested annually for suitability for culturing bacteria using the Bio-suitability test in Standard Methods method 9020 (APHA 1995).

Glassware is tested annually using an inhibitory test, according to Standard Methods method 9020 (APHA 1995). Glassware is spot-checked monthly using a bromthymol blue (BTB) solution to insure that the glassware pH is neutral, and the cleaning procedures do not inhibit bacterial growth.

Bacterial sample containers are prepared in lots and each lot is checked for sterility. If the tested container does not pass the sterility test, then all containers from the associated lot will be re-sterilized and re-tested.

Upon receipt of membrane filters from suppliers, Aquatec performs sterility checks on each lot number and record the results in the permanent logbook.

6.5.1.2 Positive and Negative Controls

One positive control, one negative control, and two rinse-blanks (at beginning and end of daily filtration) are analyzed with each daily set of coliform and *E. coli* samples (at least every 10 samples). The negative controls are conducted using the dilution water appropriate for the test. This dilution water is typically sterile phosphate rinse buffer or saline phosphate rinse buffer.

The laboratory positive and negative controls consist of the target bacteria for the specified test, such as *Escherichia coli*, *Pseudomonas sp.* or *Enterobacter sp.*, which

are routinely maintained in the laboratory. Positive controls are diluted to an appropriate concentration and are carried through the entire analysis to insure that proper culture conditions are maintained.

If the results of the negative or positive control samples indicate contamination or culture problems, then all affected samples will be re-sampled and reanalyzed.

6.5.1.3 Performance Studies

Twice annually Aquatec participates in a Proficiency Test called MicrobE, in support of our drinking water certification and NELAC requirements. Standards for this program are currently obtained from a commercial supplier (Environmental Resource Associates, ERA). Our results are submitted to ERA within the timeframe specified for the program. Also, the evaluation report will be made available to appropriate regulatory authorities, upon request.

6.5.1.4 Bacterial Verification

At least 10 percent of typical and atypical colonies on mTEC will be verified monthly using an approved method from EPA Method 1103.1. A magnification lens (10X – 15X) is used with fluorescent lighting for counting coliform and *E. coli* colony-forming units.

For gram-negative bacteria stock control cultures are verified annually for purity of culture using commercially-prepared test packs (e.g., Enterotube). If a stock culture fails verification, a new stock culture will be ordered and verified.

6.5.2 Toxicity Testing

6.5.2.1 Standard Reference Toxicant Testing

The sensitivity of the lineages of all test organisms used in the toxicity testing studies are routinely evaluated using in-house reference toxicants. Test organism responses (LC50 for acute tests; IC25 for chronic tests) are compared to specified tolerances using control charts to plot the most recent 20 values. While many organisms used for toxicity testing are purchased from reliable suppliers, any fresh or saltwater test organisms cultured in our laboratory are maintained under the recommended environmental conditions and monitored daily by laboratory personnel.

Control limits for standard reference toxicant tests are calculated in terms of multiple standard deviations from the mean value (e.g., mean LC50 or IC25). control limits are set at ± 2 standard deviations. Limits are established after a minimum of five data points depending on the analysis. Once established, the control limits are updated whenever more data points are generated, and the control chart is maintained using the

most recent 20 values.

These control limits, once calculated, are used to monitor the quality of test organisms and laboratory techniques. When analysis of a standard reference toxicant is completed the quality control data is reviewed and evaluated against these limits (most recent 20 values).

The following frequency is suggested for SRT testing:

- *Americamysis bahia* (commercially supplied): acute SRT concurrent with client tests; chronic SRT concurrent with client tests.
- *Arbacia punctulata* (in-house cultures): chronic SRT concurrent with client testing.
- *Ceriodaphnia dubia* (in-house cultures): modified acute and chronic SRT monthly;
- *Pimephales promelas* (commercially supplied): modified acute and chronic SRT monthly;
- *Menidia beryllina* (commercially supplied): acute SRT concurrent with client tests; or modified acute and chronic SRT quarterly.
- All other species: concurrent with client tests.

SRT test data are entered into the TOXIS2 statistical program and the appropriate acute or chronic values generated. The value is entered into the appropriate cumulative control chart specific for the species and reference toxicant. Acceptance is judged on the criteria of control response and control limits. If the value falls outside the control limits the reference toxicant preparation procedures should be re-evaluated.

If an error in preparation is detected, the test should be repeated whenever possible. If no technical errors are detected, the sensitivity of the organisms may be beyond the bounds of acceptability and related client-based toxicity tests may require qualification or reanalysis with new samples. Statistically, one in twenty tests may, by chance alone, fall outside of the control chart boundaries.

6.5.2.2 US EPA DMR WET

Aquatec also participates in the annual US EPA DMR WET program, which as recently been privatized. Unknown reference standards are obtained from a commercial supplier (currently ERA) and tested (acute and chronic toxicity tests) in our laboratory. Our DMR WET results are submitted to ERA and to participating NPDES clients within the timeframe specified for the program.

6.5.2.3 Control Responses and Environmental Conditions

In accordance with toxicity testing methods, each test concentration and control samples are analyzed in replicate. A control sample consisting of the dilution water (for effluent tests) or control sediment (for sediment tests) is included with each concurrent

test or group of tests. If the response of the control test organisms is outside control limits, the test conditions are scrutinized for out-of-control situations. The response of the control test organisms and the test conditions are reported with each toxicity test. The interpretation of the response and test conditions may influence the final report. If the test is judged by the Laboratory Manager and/or Director as unacceptable due to organism response and/or laboratory conditions, the test results will be rejected and Aquatec will request a new sample for analysis or retest the same sample depending on sample holding time limits.

When environmental conditions for a toxicity test are outside the range suggested by test protocols, the results of the test should be reported with appropriate qualifiers describing the out-of-range condition. The acceptability of the test will be dependent on the degree of departure from prescribed conditions and the objectives of the test. In some cases, the Laboratory Manager and the Director will review the conditions to determine acceptability. In other cases, appropriate regulatory agencies may be contacted to provide additional guidance regarding test acceptability.

6.5.2.4 Organism Identification

Aquatec cultures some organisms and purchases many others from established and reliable commercial suppliers. The Laboratory Manager and analysts are trained, through experience, to recognize the species commonly used for testing. Purchased organisms are shipped with paperwork provided by the supplier. This paper record includes the organism identity and culturing conditions of the organism. Species cultured at Aquatec (e.g., *Ceriodaphnia dubia*, *Daphnia pulex*, *Arbacia punctulata*) are identified annually.

6.5.2.5 Lighting Cycles

Light intensity shall be maintained as specified in the method SOPs or manuals. Measurement and documentation of light intensity should be performed at least annually. Photoperiods should also be maintained as specified in the method SOPs or manuals and be observed for proper function on a quarterly basis.

6.5.2.6 Toxicity Test Methodology

The methods used for toxicity tests are based upon protocols outlined in US EPA , ASTM, or US Army Corps of Engineers publications (See Section 5.5 of this QAPP). In some instances, these protocols may be modified by State-specific or Project-specific requirements. Our internal SOPs are used to define the general procedures, conditions, and acceptance criteria used for the tests performed at Aquatec. NELAC-specific SOPs describe the toxicity tests performed within the parameters of NELAC Accreditation. Also, reports submitted to our clients contain a detailed summary of the method or protocol followed for that particular test. Any deviations from the reported method are noted as qualifiers within the report (See Section 5.8 for additional

information regarding reporting of toxicity testing results.).

6.6 SOFTWARE QUALITY CONTROL

Aquatec currently employs two commercial statistical programs for evaluation of toxicity data. TOXIS2 is used primarily for whole effluent toxicity (WET) test analysis, while TOXSTAT is used primarily for sediment or soil toxicity analysis.

Part of our assessment of the performance of these programs is accomplished through our standard reference toxicant program, whereby acute or chronic values generated from actual tests performed in the laboratory should fall within the Control Chart limits.

Also, as part of our annual Quality System Review, Aquatec will enter a known data set into these programs. For example, a data set from US EPA toxicity testing guidance documents may be used to assess the function of our toxicity software programs (TOXIS2 and TOXSTAT). This annual assessment of the performance of statistical packages will be documented, reviewed, and filed. If acute or chronic values match the published values (within the confidence limits), the statistical package will be assessed as being acceptable. Our TOXIS2 statistical package is also tested annually through the US EPA DMR Quality Assurance study because this statistical package is used to generate the values submitted for this study.

In-house statistical or calculation programs developed by Aquatec will also be assessed annually completing an independent computer-based or manual calculation to verify that the program is functioning properly.

6.7 AUDITS

6.7.1 Audits from Regulatory Agencies

As a participant in state and federal certification programs and the New Hampshire Environmental Laboratory Accreditation Program (NELAC), Aquatec may be audited by representatives of these agencies. Audits typically focus on laboratory conformance to the specific program protocols for which the lab is seeking certification or accreditation. Auditors are likely to review sample handling and tracking documentation, traceability (of chemicals, standards, and reagents), analytical methodologies, analytical supportive documentation, and final reports. Audit findings from regulatory agencies are formally documented. Aquatec will prepare a written response to audit findings and will develop a plan to address any required corrective actions. The written response will address each item presented in the audit report and will be submitted to the auditing authority.

6.7.2 Internal Audits

NELAC standards require a documented annual internal audit. Internal technical audits

and annual Quality System review will be administered by the Director/Quality Assurance Officer. The format for the internal audit will be modeled on guidelines of EPA/600/4-90/031 (*Manual for the Evaluation of Laboratories Performing Aquatic Toxicity Tests*) or other self-audit formats, with a focus on the requirements of NELAC standards. The audit will involve a "Check List" format, similar to that suggested by EPA/600/4-90/031 "Pre-Survey Form".

The findings of internal audits will be formally documented. The Laboratory Manager will have the responsibility for resolving points at issue or for effecting necessary changes to the laboratory's practices in a timely manner. The target time frame for addressing corrective actions resulting from the internal audit is one month from the completion of the internal audit report.

The internal audit program will focus on the following areas:

- Maintenance of SOPs in company and NELAC-acceptable format.
- Maintenance of training records.
- Maintenance of notebooks or data packages for each project.
- Maintenance of instrument maintenance and calibration records.
- Evaluation of reagent labeling and tracking.
- Evaluation of standards control records (e.g., laboratory water monitoring).
- Evaluation of sample handling procedures.
- Evaluation of data handling and storage procedures.

6.7.3 Audit Response

The laboratory will respond with corrective action to the audit findings and recommendations of the regulatory agencies so that the goal of certification for a particular program can be granted.

If a recommendation is related to revising particular documents to meet the standards required by the audit, then the revisions will be made and a copy of the revised document(s) will be forwarded to the auditor, if required.

If a recommendation is related to a laboratory procedure (for example, error correction), then the recommendation will be communicated to the laboratory personnel informing them of the correct procedure and a record of this communication will be submitted to the appropriate representatives of the regulatory agency. In conjunction with this, the QAPP or SOPs may be annotated to incorporate the required corrections. In this case, these documents (QAPP and SOPs) will be formally revised during the annual Quality Systems review. A copy of the annotations or the revised QAPP or SOPs will be made available to the auditor upon request.

6.7.4 Data Audits

The Director/QA Officer will audit a representative report and associated data package at least quarterly. This will involve reviewing a current report/data package or one that has been or will be submitted to a client. If an error is detected that affects the reported outcome of the analysis (for a report already submitted), the client will be notified immediately and a revised report will be submitted to the client. A written narrative will accompany the corrected report notifying the client of the error. The results of the data audit will be documented.

If an audit (either internal or external) indicates that a client's analytical results are questionable, the client will be notified and a decision will be made as to whether a report revision or possibly re-analysis of samples is required. Aquatec will then work with the client and make every attempt to resolve any issue(s) needing attention.

If substantive errors are detected by an internal data audit, the Director/Quality Assurance Officer will conduct a follow-up audit, to verify that corrective action has been implemented. Observations made during this follow-up audit will be made available to the appropriate representatives of the regulatory agency upon request.

6.7.5 Quality System Review

On an annual basis, management (Director/QA Officer and Lab Managers) will conduct a documented review of the Quality System. The purpose of this review is to ensure the suitability and effectiveness of its program, to ensure that certification standards are met, and to provide opportunity for improvements. The review includes the following topics:

- Follow-up on decisions made in meetings;
- Evaluate reports from audits by clients and regulatory agencies;
- Evaluate reports from internal audits;
- Evaluate results of proficiency studies;
- Evaluate results of water quality monitoring;
- Evaluate corrective actions from the past year and implement;
- Evaluate details of complaints from clients and their resolutions;
- Evaluate training goals and objectives;
- Evaluate staff, facility and equipment resources;
- Evaluate future plans and goals; and,
- Revise the QAPP, Employee Manual, and SOPs, as needed.

6.7.6 Performance Audits

The laboratory participates in external laboratory check sample or proficiency programs as a means for examining overall laboratory performance as well as to qualify for various federal and state certification programs. The following external or inter-

laboratory check sample programs are employed to demonstrate analytical proficiency for purposes of monitoring overall laboratory proficiency or to provide proof of acceptable performance for certification by outside agencies or regulatory bodies:

USEPA Discharge Monitoring Report Quality Assurance Study (DMR QA)

This program consists of unknown samples (“simulated effluents”) for aquatic toxicity testing. This program provides a measure of accuracy, because results are comparative on a nation-wide scale. The laboratory receives a report detailing acceptability of the reported results. The EPA report is then submitted to all clients requesting aquatic toxicity testing services. If any results fall within the “unacceptable” category, a review of the test data is performed, and a response and corrective action report is sent to the administrator of the program and also to any clients required to receive the response.

State of Vermont Department of Health: This program consists of bi-annual audits of the Microbiology Laboratory associated with certification in the State of Vermont for microbiological analyses.

Microbiology Proficiency Test: Twice annually Aquatec participates in a Proficiency Test called MicrobE, in conjunction with drinking water certification and NELAC requirements. Standards for this program are currently obtained from a commercial supplier (Environmental Resource Associates, ERA).

State of New Hampshire Department of Environmental Services Environmental Laboratory Accreditation Program: Aquatec has submits required documents for annual renewal of NELAC accreditation. The current Quality Systems is reviewed to address any deficiencies resulting from accreditation audits.

6.8 CERTIFICATIONS

A listing of Aquatec’s certifications and accreditations is found in Appendix D.

6.9 CORRECTIVE ACTION

To the extent possible, samples are reported only if all quality control measures are acceptable. If a quality control measure is found to be out of range or out of control, and the data are to be reported, all samples associated with the failed quality control measure shall be reported with the appropriate laboratory defined data qualifiers (NELAC 2003, Section 5.4.10.6).

When deficiencies or “out-of-control” situations exist, the Quality Systems program provides a means for detecting, documenting, and correcting these situations. Samples analyzed during out-of-control situations may be reanalyzed prior to reporting of results or re-sampling and re-testing may be required. There are several levels of “out-of-control” situations that may occur in the laboratory during analysis.

Documentation of out-of-control situations may be limited to notations made on Chain-of-Custody forms (e.g., for samples received) or laboratory bench sheets for specific test methods, when appropriate.

A Corrective Action Report, (Appendix E), if generated, may be completed and filed for situations that represent either singular or systematic QA or QC problems. Samples broken in transit to the laboratory, missed holding times, suspect standard/reagent lot, non-compliant calibration, or software problems could be documented on these reports. The culmination of all corrective action reports for a particular sample set will be used to assist the project director in preparing a narrative to accompany the sample delivery group.

The individual who experienced the event needing corrective action will initiate the action by documenting the events on bench sheets that become a part of the permanent record for that analysis or by completing a Corrective Action Report describing the events. For example, if samples were broken in transit, then the employee unpacking the cooler would document the event on the Chain-of-Custody form or initiate the Corrective Action Report. If the person initiating the report is uncertain as to what would constitute appropriate corrective action for the situation, then the Lab Manager or Director should be consulted for resolution.

Copies of all corrective action documentation (including QA reports: control performance and qualifiers) will be submitted to Management, the Director/Quality Assurance Officer, for review. The corrective action reports or documentation will also be filed with the project documentation.

6.9.1 Bench-level Corrective Action

While some events in the laboratory require corrective action, they may not be viewed as “out of control” events. An example of this would be where a miscount of organisms has been made in a toxicity test replicate. The requirement for correcting test data is described in Section 7.1.

An event potentially requiring corrective action for an “out of control” event requires that the analyst write a notation on the bench sheets or attached paperwork describing the event. The analyst must also notify the Laboratory Manager that a “lab error” has occurred. An example of this, in the toxicity laboratory, would be an error resulting in spilling a test replicate. The Manager and/or Director must then make a decision as to whether the toxicity test should continue (with reduced replication at one treatment level), or whether the test should be repeated. The final decision as to the impact of the event may not occur until the test and the statistical analysis of the data has been completed.

Other corrective action procedures are often handled at the bench level. If an analyst

finds a non-linear response during calibration of an instrument, then the instrument is recalibrated before sample analysis commences. The instrument will also be recalibrated if reference or internal standard results are outside of specified tolerances. Problems are often corrected by a careful examination of the preparation or calibration procedure or instrument sensitivity. If a problem persists, it is brought to the attention of Lab Manager or Director. If an analyst is aware of an unusual event that could affect the test result or data quality (e.g., spilled test replicate, unusual mortality) during an analysis the analyst must document the event and bring it to the attention of the Laboratory Manager and/or the Director.

6.9.2 Management-level Corrective Action

If resolution at the bench level was not achieved or a deficiency is detected after the data has left the bench level, corrective action becomes the responsibility of the Department Manager or Director. A decision to reanalyze the sample or report the results is made depending on the circumstances of the sample and analysis. Documentation procedures for sample re-analysis are initiated at this point if necessary.

6.9.3 Receiving-level Corrective Action

If discrepancies exist in either the documentation of a sample or its container, a decision must be made after consulting with the appropriate management personnel. Examples of discrepancies include: broken sample containers; inappropriate containers; improper preservation; improper temperature; or, extended holding times. In these cases, corrective action may involve the contacting the client to resolve the problems. All resolutions must be fully documented and filed with the sample results. Also the results will be reported as “qualified”.

6.9.4 Statistical Events

An out-of-control statistical event is defined as data exceeding control limits, unacceptable trends detected in the charts, or unusual changes in the instrument detection limits. When these situations arise, it is brought to the attention of the Department Managers or Director who will initiate the appropriate corrective action. Corrective action may take the form of instrument maintenance, recalibration, or re-analysis of the sample.

6.9.5 Client or Data Validation Feedback

If a client or data validator has a question or finds a deficiency concerning the data submittal, the Laboratory Manager or the Director, is responsible for communicating and implementing the corrective action to laboratory personnel. The analytical results and all supportive documentation will be re-evaluated. Should a reanalysis be necessary, it may be initiated if the sample is still available and within prescribed

holding times. Since microbiology samples and effluent samples have short holding times, re-analysis of stored samples might not be practical and re-sampling might be necessary. If revisions to the report are necessary, the corrections are integrated into a revised report that is submitted to the client.

Hard copies and revised electronic deliverables (where applicable) will be re-submitted to the client or a data validator when appropriate. In some instances, clients request that additional sample handling information, recalculations, or qualitative judgments be provided. In this case, resubmission of the data may not be necessary unless a problem is found.

All customer complaints will be documented and the report filed in the complaints file within the administration office. This report will have at a minimum: Client contact information; 2) Type(s) of analyses; 3) Complaint; 4) Documented Resolution(s); 5) Person handling the complaint. Customer problems that persist in the laboratory are to be communicated to the Director and where appropriate, corrective action will be initiated.

6.9.6 Cause Analysis

When nonconforming work or departures from policies or procedures have been identified, the procedure for corrective action will start with an investigation to determine the cause. Action(s) most likely to eliminate the problem and prevent recurrence should be implemented. Use of the Corrective Action Report (Appendix E) will provide a format for identifying, describing, and resolving corrective action needs.

7.0 INVENTORY PROCEDURES FOR REFERENCE STANDARDS, REAGENTS AND CONSUMABLE MATERIALS

In conformance with NELAC requirements (Section 5.5.6.4 of the 2003 Standard), Aquatec will maintain a record of purchased reagents that will be documented as to source (linked by the purchase order number), receipt date, lot number, expiration date (where applicable), and disposal date. This information is to be recorded in the Reagent Log that is located at the reception area. Any certificates of analysis must be stored in the file labeled "Reagents: Certificates of Analysis" located in the project files for the current year. Also, each commercial reagent is required to carry a label with the following information completed:

Commercial Reagent Label

Date Received:	Source:
Lot #:	Date Opened:
Storage:	Expiration date:

Each laboratory-prepared standard or solution in the laboratory must be linked to the original reagent through the lot number of the original reagent(s). The lot numbers of the original purchased reagents are recorded in the Reagent Preparation Log at the time of preparation. Also, each prepared reagent or will carry the following label:

Prepared Reagent Label

Description / Use:	
Date prepared:	Lot #:
Storage:	Expiration date:

For Laboratory Reconstituted Waters, the reagent lot number information must also be included in the Water Preparation Log.

Reference toxicant solutions must also carry linkage to the lot numbers of stock solutions. The lot number of reference toxicant solutions may be the sample number, assigned during login.

8.0 LABORATORY DOCUMENTATION

Employees generating data are required to be included in the log of names, initials, and signatures associated with this version of the QAPP (Appendix A). The initials (or variations) should match the initials (or variations) that the individual uses to document observations.

Workbooks, bench sheets, instrument logbooks, and instrument printouts, are used to trace the history of samples through the analytical process and to document and relate important aspects of the work, including the associated quality controls. All logbooks, bench sheets, instrument logs, and instrument printouts are part of the record of the laboratory work. Completed workbooks and/or bench-sheets, are submitted as part of the data package for review. These are stored, with the final report, in the appropriate project file. Active Instrument logbooks are stored in the laboratory. At the end of the calendar year the instrument logbooks are filed with project files for that year and maintained as described in Section 5.8.

8.1 DATA RECORDING ERRORS

Errors in entry are to be crossed out in indelible ink with a single stroke and corrected without the use of white-out or by obliterating or writing directly over the erroneous entry. All corrections are to be initialed and dated by the individual making the correction. In some cases (e.g., data used to generate end points to an analysis),

justification for the data change should be documented. Pages inserted into permanent logbooks are to be stapled or taped to a clean, bound page. The analyst's initials are to be recorded in such a manner that the initials overlap the inserted page and the bound page. Pages of logbooks which are not completed (as part of normal record keeping) should be completed by lining out unused portions.

8.2 STANDARD OPERATING PROCEDURES

Standard Operating Procedures (SOP's) contain the basic procedures and practices the laboratory uses to complete requested methods or analyses. These procedures provide a basis for training new employees and for providing consistency in the performance of the procedure. SOPs may contain proprietary information and as such are controlled documents. Method-specific SOPs for NELAC-accreditation should be formatted following the guidance of the NELAC standards (NELAC 2003 Standards, Section 5.5.4.1.2). The following elements are to be incorporated into SOPs pertaining to NELAC-accredited methods:

1. identification of test method
2. detection limit;
3. scope and application;
4. summary of test method;
5. definitions;
6. interferences;
7. safety;
8. equipment and supplies;
9. reagents and standards;
10. sample collection, preservation, shipment, and storage;
11. quality control;
12. calibration and standardization;
13. procedure;
14. calculations;
15. method performance;
16. pollution prevention;
17. data assessment and acceptance criteria for quality control measures;
18. corrective actions for out-of-control data;
19. contingencies for handling out-of-control or unacceptable data;
20. waste management;
21. references;
22. tables, diagrams, flowcharts, and validation data.

Some of the above-listed elements may not apply to a particular SOP. When this occurs, the words "Not applicable" may be written for that element or section. Also, SOP sections may be interlinked by reference to other SOPs.

Also, some laboratory procedures are described in SOPs designed as brief and very clear instructions (e.g., glassware cleaning, instrument operation) to laboratory analysts. These SOPs are not required to contain all of the above-listed elements, but should be in a similar format. In cases where a formal SOP is not in place, manufacturers operation and maintenance manuals are an acceptable substitute.

The principal purpose of the SOP is to provide a description of internal analytical procedures so that uniform methods are applied. The SOP provides information on the preparation of standards or reagent solutions, instrumental functions and operation, and analytical procedures, and quality control criteria, etc. and is especially beneficial during the training process for new personnel. Nearly every operational procedure in the laboratory should have a supporting SOP.

SOP's are written procedures often reflecting standardized or published methods and protocols (e.g., Standard Methods, instrument operation instructions, or EPA-600 methods). While SOPs will carry the basic information required to accomplish a specific procedure, the SOP may not contain all of the information presented in the published document. The function of the SOP at Aquatec is to provide the analyst with the essential information required to be proficient with that procedure. Also, some Aquatec SOPs are written to describe an "in-house" procedure that may have been developed here, so that methods developed in-house will be documented and repeatable. A master copy of an SOP bears the approval signatures of the Director and the Laboratory Manager responsible for the procedure.

As an important element of our Quality System, our SOPs incorporate a signature page for evidence of reading, understanding, and agreement to follow by each analyst. The signature page refers to a single SOP and contains the printed name, signature, and date read by the analyst for that SOP.

SOPs are controlled internal documents. This means that they are not for general distribution outside of the laboratory. Appropriate regulatory authorities are allowed to view SOPs, with the Director's approval. If a client requests a copy of an SOP, an electronic or paper copy may be made available, with the Director's approval.

SOP's are reviewed on an annual basis as part of the annual Quality Systems review. Due to the large volume of SOPs carried by Aquatec, it is not practical to do SOP review in a short time-frame, therefore, throughout the year SOPs are reviewed and updated if needed. Whenever an SOP is revised, a new revision number will be assigned and that version will be stored electronically in a "Final SOPs" folder. Also, paper copies of the most recent version of SOPs will be stored in a notebook in the laboratories – available to all laboratory analysts. The prior electronic version will be archived electronically in a "Expired SOPs" folder and at least one paper copy of the prior version will be archived in a "Expired SOPs" hard copy folder. Any SOPs in the developmental phase will be stored electronically in the "Draft SOPs" folder until it is finalized and then moved into the "Final SOPs" folder.

If a minor amendment or clarification is needed for a particular SOP, the amendment may be accomplished through a hand-written annotation on the lab copy of the SOP. Analysts will be required to read and initial the annotation(s) to acknowledge the revision. During the review cycle for SOPs, or if revision is substantial, this annotation (if appropriate) should be incorporated into the revised final SOP and the revision number (next higher number) should be updated.

8.3 DOCUMENT CONTROL

Security and control of documents is necessary to ensure that confidential information is not distributed and to make sure that active copies of a given document are from the latest applicable revision. Controlled documents have a header placed in the upper right hand corner of each page. This header provides enough information to unambiguously identify each page as part of a single compiled document. At a minimum, the following information will be supplied:

Document Name:
Revision Number:
Revision Date:
Page ____ of ____

The Director/QA Officer controls the following documents for Aquatec Biological Sciences, Inc.:

- Quality Assurance Program Plan
- Standard Operating Procedures
- Employee Manual
- Chemical Hygiene Plan

9.0 STORAGE OF RECORDS

9.1 DATA STORAGE

Level I, II and III Data - Worksheets containing the supportive documentation for Level I, II and III reported analyses are stored by project in file cabinets for at least one year. After this time period, the worksheets, project information, and data are maintained in metal filing cabinets or are archived for a minimum of five (5) years.

Level IV, V Data - The completed data package including supportive documentation for level IV and V data are stored by project in file cabinets for at least one year. After this time period, the worksheets, project information, and data are maintained in metal filing cabinets or are archived for a minimum of five (5) years. If requested, data can be stored for longer periods of time. Requests for extended storage should be communicated at the beginning of the project if possible.

Calibration records, test organism records, laboratory water analysis data, DMR reports,
Aquatec Biological Sciences, Inc.

reference toxicant data, etc. are stored for a minimum of five (5) years in the project files for that year.

In the event of transfer of ownership or other event resulting in the termination of Aquatec Biological Sciences, Inc. as a business, records will be maintained or transferred according to clients instructions or are archived in a secure and announced location for a minimum of five (5) years from the date of the analysis or project work.

10.0 SAMPLE DISPOSAL

Method-specific SOPs outline sample disposal, pollution prevention, and waste management procedures followed at Aquatec. Appendix B of this QAPP provides additional guidance regarding storage and disposal of samples.

Unused sample portions and extracts are stored appropriately (usually refrigerated or frozen) for the amount of time specified by contractual obligations and project specifications after the submission of the data package. After their storage period expires, the samples may be removed from the refrigerator/freezer and stored in a secured storage area. Unused sample portions removed from the refrigerators may be drummed, packed, and disposed of as either non-regulated or hazardous waste depending on the results of the analyses.

Aquatec has a hazardous waste storage area containing 55-gallon drums for sorting and packing lab waste. The waste is packed according to specifications outlined in Federal Regulations and Department of Transportation regulations. Periodically, the waste is transported by an approved hazardous waste hauler, to an appropriate disposal/treatment facility.

The following general guide-lines apply to sample disposal:

NPDES effluents and receiving waters: unused sample may be discarded after one week following test completion (unless otherwise directed). These samples are discarded after disinfection (with bleach) to normal drainage. Samples that are shown to be potentially highly toxic (e.g., LC50 less than 10%) should be transferred to the aqueous waste drum for disposal by a waste disposal firm.

Sediments and Soils: Sediments and soils may be discarded 30 days after the report has been submitted to the client. In some instances, clients request an extended storage time for these samples. Residual sediments and soils are discarded in the solid waste drums for disposal by a waste disposal firm.

Reference toxicants: Whenever possible, Aquatec uses reference toxicants (sodium chloride and potassium chloride) that are not harmful to humans in routine use and can be disposed of through normal drainage. Copper sulfate is used as the reference toxicant for the sea urchin, *Arbacia punctulata*, fertilization and embryo development tests. Solutions made up with this material are discarded to the aqueous waste drum.

Formalin: Small volumes of formalin waste (e.g., less than 1 L) are transferred to the aqueous waste drum. Formalin waste from preserved macroinvertebrate samples are neutralized with sodium sulfide before disposal to regular waste

Acids and bases: These solutions are highly diluted before release to the normal sink drainage.

Acetone waste: Waste acetone is transferred to a specific “Acetone Waste” drum, for disposal by a waste disposal firm.

Microbiology waste: Unused samples are discarded after disinfection (with bleach) to normal drainage. Unused solid waste from analyses (media, petri dishes and contents) are autoclaved before disposal to regular waste.

**QUALITY ASSURANCE
PROGRAM PLAN
March 2006
Revision 9**

LOG OF NAMES, INITIALS, AND SIGNATURES

**Certification of reading and understanding and agreement to follow
the current version of the Aquatec Biological Sciences, Inc. QAPP.**

Printed name	I agree, to the best of my ability to follow the requirements of this QAPP Signature	Initials used to document observations	Date
Phiip Downey			
John Williams			
Jennifer Gallant			
Katrina Simpson			
Kaitlyn Koch			
Stuart Randall			
Karen Downey			

APPENDIX A: RESUMES

Resumes

Resumes for the following specific personnel are included in the following pages.
Additional resumes are available upon request.

Philip C. Downey, Ph.D. -

Director
Manager, Microbiology,
Quality Assurance Officer
Manager, Ecology and limnology

John W. Williams B.S.

Manager, Environmental Toxicology

Jennifer Gallant, B.S.

Microbiology
Macroinvertebrate taxonomy
Environmental toxicology support
Limnology analyses

Katrina Simpson, B.S.

Environmental Toxicology
Microbiology support

Kaitlyn Koch

Environmental Toxicology
Macroinvertebrate sample preparation

Stuart Randall, B.S.

Zooplankton taxonomy

Brief resume of key person, specialists, and individual consultants anticipated for this project.	
a. Name & Title: Philip C. Downey Director	Dr. Downey has also conducted mussel bioaccumulation studies for NPDES discharges into the Massachusetts Bay. Bioaccumulation Studies - Project Director for freshwater and saltwater bioaccumulation studies which included both vertebrates and invertebrates. Target analytes included heavy metals, Polynuclear Aromatic Hydrocarbons (PAH), pesticides, and Polychlorinated byphenyls (PCB). He was responsible for statistical analyses and reporting.
b. Project Assignment: Director	
c. Name of Firm with which associated: Aquatec Biological Sciences	Ecological Assessments - Feasibility studies and biological evaluation of a new State of Vermont fish culture facility. He provided biological evaluation for the quality of intake water, the fish culture station design, and proper operation and modeling of effluent water quality. These projections were used to evaluate the possible effects of hatchery discharge on lake biota.
d. Years experience: With This Firm 10 With Other Firms 17	
e. Education: Degree(s)/Year/Specialization Ph.D./1982/Fisheries M.S./1978/Fisheries B.S./1975/Fisheries B.S./1975/Biology	Fish Culture Assessments - Evaluation of the State of Vermont's five existing fish culture stations. This evaluation examined the long-term culture program goals, management alternatives, and existing water supplies at the facilities. He was the author of the final report which provided recommendations for number, and species to be produced at each facility as a concept of renovations for each fish culture station to optimize fish production.
f. Active Registration: Year First Registered / Discipline 1990/Certified Fisheries Scientist (CFS) 1992/Past President AI Chapter American Fisheries Society	Expert Witness Testimony - Provided expert witness testimony in a deposition taken for a lawsuit filed in the U.S. Federal court. His testimony was on a study conducted to evaluate the potential impact of a paper mill. Conducted a review of an EPA RI/FS on a major Superfund site in Vermont as part of a Superfund Technical Advisor Grant (TAG).
g. Other Experience and Qualifications relevant to the proposed project: Dr. Downey is the Director, with over 16 years of experience in developing, planning, and implementing complex environmental studies. He is currently responsible for the management of the microbiology laboratories. His experience in managing AI biological projects includes ecological assessments, bioaccumulation studies, toxicological assessments and microbiological analyses. Representative Project Experience: Mussel Bioaccumulation Studies - Project director of MWRA bioaccumulation studies to assess contaminant body burdens at Deer Island and the new outfall since 1991. He has presented numerous presentations on this work at scientific meetings, Massachusetts Bay Symposia and MWRA workshops.	Toxicological Assessment - As part of the Superfund contract Laboratory Program (CLP), whole water, elutriate and solid phase samples were analyzed for projects as part of RI/FS studies being conducted by the EPA. As director, he was responsible for the laboratory analysis and report completion for projects. Microbiological Analysis - Dr. Downey conducted studies of industrial ultra-pure water using epifluorescence microscopy and plate count techniques. These studies included troubleshooting, statistical data analyses and recommendations. Publications: Hall, Maury and Philip Downey. 1994. Selected Organic and metal Contaminant Bioaccumulation in Mussels Deployed in Boston Harbor and Massachusetts Bay. Poster presented at the ninth annual Boston Harbor/Massachusetts Bay Symposium, Boston, MA.

Brief resume of key person, specialists, and individual consultants anticipated for this project.	
a. Name & Title: John W. Williams Toxicity Laboratory Manager	<p>Representative Project Experience:</p> <p><u>NPDES Compliance Biomonitoring:</u> Mr. Williams manages whole effluent toxicity testing (marine and freshwater) programs for numerous industrial and municipal wastewater discharge facilities throughout U.S. EPA Region I and New York. Mr. Williams is responsible for coordinating client testing schedules, supervising testing activities, data review, and reporting.</p> <p><u>Toxicity Identification Evaluation (TIE):</u> Mr. Williams is responsible for the technical aspects and reporting of TIE investigations. Several Phase I and partial Phase I TIEs for municipal and industrial discharges have been completed.</p> <p><u>Sediment and Soil Toxicity Assessment:</u> Mr. Williams is responsible for the completion of whole sediment toxicity and bioaccumulation analyses of samples from Superfund or proposed dredged material sites using marine, estuarine, freshwater, and terrestrial species.</p> <p><u>Chemical Product Testing:</u> Mr. Williams was responsible for the toxicity characterization of a series of polymer products from several manufacturers. Chronic toxicity characterizations were completed using the water flea, <i>Ceriodaphnia dubia</i> as the test species.</p> <p><u>Publications and Presentations:</u> Battelle (J.W. Williams author). 1988. "Final Report, XXX Insecticide: Acute toxicity to grass shrimp (<i>Palaemonetes pugio</i>) and inland silverside minnow (<i>Menidia beryllina</i>) in a flow through system". FIFRA Guideline 72-3. Carr, R.S., J.W. Williams, and C.T.B. Fragar. 1989. "Development and evaluations of a novel marine sediment pore water toxicity test with the polychaete, <i>Dinophilus gyrociliatus</i>". Environ. Toxicol. Chem. 8: 533-543. Jop, K.M., J.W. Williams, and R.B. Foster. 1990. "Toxicity evaluation of the proposed secondary and primary effluents discharged to Massachusetts Bay". Bull. Environ. Tox. 45: 399-407. Williams, J.W., M.P. Hall, P.C. Downey. 1999. "On-site toxicity assessment using the sea urchin, <i>Arbacia punctulata</i>. Society of Environmental Toxicology and Chemistry, poster presentation.</p>
b. Project Assignment: Aquatic Toxicity Testing	
c. Name of Firm with which associated: Aquatec Biological Sciences, Inc.	
d. Years experience: With This Firm 10 With Other Firms 18	
c. Education: Degree(s)/Year/Specialization B.S./1968/Biology-Fisheries	
d. Registrations: Year First Registered / Discipline 1975/NAUI SCUBA Instructor 1993/Society of Environ. Tox. And Chemistry 1993/New England Association of Environ. Bio.	
g. Other Experience and Qualifications: Mr. Williams is responsible, as manager of the Toxicity Testing Laboratory, for scheduling and directing all work conducted by Aquatec's toxicity testing laboratory. He draws upon a broad range of experience to manage testing activities, supervise and train laboratory staff, maintain client relations, submit reports, and develop new technical areas for Aquatec. Mr. Williams has over ten years experience in the area of aquatic and whole sediment and soil toxicity testing. His experience includes both freshwater and marine testing under NPDES, U.S. EPA, ASTM, FIFRA, TSCA, and ACOE regulation. He also has experience in the identification of marine invertebrate species, supervising field collection operations and directing SCUBA operations.	

Brief resume of key person, specialists, and individual consultants anticipated for this project.	
e. Name & Title: Jennifer J. Gallant Microbiologist	<p>Chlorophylls: Ms. Gallant is responsible for conducting chlorophyll <i>a</i> analyses, including data entry.</p> <p>Inorganic chemistry: Ms. Gallant is an analyst for several inorganic chemistry parameters including alkalinity, hardness, ammonia, and nutrient analyses such as ortho-phosphorus, nitrate, nitrite, and total phosphorus. Ms. Gallant prepares chemical reagents for conducting chemistry analyses and is responsible for maintaining and ordering stocks of chemical reagents.</p>
f. Project Assignment: Microbiology, Ecology	
c. Name of Firm with which associated: Aquatec Biological Sciences	
d. Years experience: With This Firm 7 With Other Firms 0	
e. Education: Degree(s)/Year/Specialization B.S./1998/Biology	
f. Active Registration: Year First Registered / Discipline	<p>Representative Project Experience:</p> <p>Microbiological testing (June 2003-Spring 2004): Ms. Gallant participated in the watershed approach for the Merrimack River project. The project involved fecal coliform, <i>E. coli</i> and fecal <i>Streptococcus</i> testing, and chlorophyll <i>a</i> analysis. She organized for the project, prepared media, performed the chlorophyll <i>a</i> analyses, and supported data entry and quality assurance.</p>
<p>g. Experience and Qualifications relevant to the proposed project:</p> <p>Ms. Gallant has five years of experience in the field of environmental testing. Her experience includes a broad range of chemistry and biological analyses. She also provides support for field sampling, and macroinvertebrate work.</p> <p>Microbiology: Ms. Gallant is responsible for all aspects of work in the Microbiology Laboratory. She conducts membrane filtration and multi-tube fermentation analyses on potable and non-potable water samples, as well as heterotrophic plate count methods. Her responsibilities also include food analyses using various AOAC protocols, Microtox, preparation of microbiological media, data entry, and quality assurance for the microbiology laboratory.</p> <p>Ms. Gallant provides support for conducting whole effluent toxicity tests (freshwater and marine) for current NPDES clients. She has also conducted sediment toxicity tests using several freshwater and marine species. She has maintained laboratory cultures of <i>Ceriodaphnia dubia</i> and <i>Chironomus tentans</i> for ongoing toxicity testing programs.</p>	

Brief resume of key person, specialists, and individual consultants anticipated for this project.	
h. Name & Title: Katrina Simpson (formerly D'Anjou) Biologist	<p>Representative Project Experience:</p> <p>Toxicity Evaluation of Sediments (2003): Participated in performing marine sediment toxicity and bioaccumulation tests in support of an US Army Corps of Engineers assessment of dredged sediments in Connecticut Harbors and Boston Harbor. Test species used included <i>Americamysis bahia</i>, <i>Menidia beryllina</i>, <i>Ampelisca abdita</i>, <i>Eohaustorius estuarius</i>, <i>Nereis virens</i>, and <i>Macoma nasuta</i>.</p> <p>Toxicity Evaluation of Sediments (2003): Participated in performing whole sediment toxicity tests in support of risk assessments at sites. The project involved testing whole sediment samples with <i>Hyalella azteca</i> and <i>Chironomus tentans</i>.</p> <p>Whole Effluent Toxicity Testing: Responsible for conducting all aspects of whole effluent toxicity tests (freshwater and marine) for current NPDES clients. Test species used include <i>Ceriodaphnia dubia</i>, <i>Pimephales promelas</i>, <i>Menidia beryllina</i>, and <i>Americamysis bahia</i>, and <i>Salvelinus fontinalis</i>.</p> <p>Bioaccumulation Evaluation of Soils (2003): Participated in the evaluation of soils collected from an US Army base on the West Coast. The test species was the earthworm, <i>Eisenia foetida</i>.</p> <p>Standard Reference Toxicant Tests: Responsible for preparing and conducting standard reference toxicant (SRT) tests on organisms used for sediment and WET testing.</p> <p>Microbiology: Experience in the microbiology laboratory includes membrane filtration and multi-tube fermentation analyses on potable and non-potable water samples. Her responsibilities also include preparation of microbiological media.</p> <p>Inorganic chemistry: Responsible for several inorganic chemistry parameters including alkalinity, and hardness. She also prepares chemical reagents for conducting chemistry analyses.</p>
i. Project Assignment: Environmental Toxicology	
c. Name of Firm with which associated: Aquatec Biological Sciences, Inc.	
d. Years experience: With This Firm 3 With Other Firms 0	
e. Education: Degree(s)/Institution/Year/Specialization St. Michael's College / 2003 / B.S., Biology	
f. Active Registration: Year First Registered / Discipline	
<p>g. Experience and Qualifications:</p> <p>Ms. Danjou has experience in the field of environmental testing. Her experience includes a broad range of biological analyses (toxicity testing, microbiology, and macro-invertebrate sorting) and supporting chemistry.</p> <p>Her toxicity laboratory experience includes aquatic whole effluent toxicity (WET) testing with freshwater and marine species. She also conducts sediment toxicity tests using several freshwater and marine species. She maintains laboratory cultures of <i>Ceriodaphnia dubia</i> and <i>Arbacia punctulata</i> for ongoing toxicity testing programs. Her responsibilities also include analytical support for the Microbiology Department.</p>	

Brief resume of key person, specialists, and individual consultants anticipated for this project.	
h. Name & Title: Kaitlyn Koch Biologist	<p>Representative Project Experience:</p> <p>Toxicity Evaluation of Sediments: Participated in performing whole sediment toxicity tests in support of risk assessments at marine and freshwater sites. The projects involved testing whole sediment samples with <i>Hyaella azteca</i>, <i>Chironomus tentans</i>, <i>Ampelisca abdita</i>, and <i>Leptocheirus plumulosus</i>.</p> <p>Whole Effluent Toxicity Testing: Responsible for conducting all aspects of whole effluent toxicity tests (freshwater and marine) for current NPDES clients. Test species used include <i>Ceriodaphnia dubia</i>, <i>Pimephales promelas</i>, <i>Menidia beryllina</i>, and <i>Americamopsis bahia</i>.</p> <p>Bioaccumulation Evaluation of Soils: Participated in the evaluation of soils collected from an Air Force base. The test species was the earthworm, <i>Eisenia foetida</i>.</p> <p>Standard Reference Toxicant Tests: Responsible for preparing and conducting standard reference toxicant (SRT) tests on organisms used for sediment and WET testing.</p> <p>Inorganic chemistry: Responsible for several inorganic chemistry parameters (e.g., dissolved oxygen, conductivity, pH, salinity).</p>
i. Project Assignment: Environmental Toxicology, Ecology	
c. Name of Firm with which associated: Aquatec Biological Sciences, Inc.	
d. Years experience: With This Firm 1 With Other Firms 0	
j. Education: Degree(s)/Institution/Year/Specialization Plymouth State University / 2004 / B.S., Environmental Biology	
k. Active Registration: Year First Registered / Discipline: N / A	
<p>l. Experience and Qualifications:</p> <p>Toxicology Laboratory: WET testing solution preparation and toxicity test monitoring for freshwater and marine species. Maintenance of laboratory cultures of <i>Ceriodaphnia dubia</i>, <i>Daphnia pulex</i>, <i>Arbacia punctulata</i>. Performs alkalinity and hardness measurements. Initiation, monitoring and maintenance, and completion of sediment toxicity tests.</p> <p>Ecology Laboratory: Macroinvertebrate sample processing and slide preparation, bone and scale preparations.</p>	

Brief resume of key person, specialists, and individual consultants anticipated for this project.	
m. Name & Title: Stuart Randall Ecology	Representative Project Experience: CDM Oklahoma Phytoplankton and Zooplankton survey (on-going)
n. Project Assignment: Ecology / Biology	
c. Name of Firm with which associated: Aquatec Biological Sciences	
d. Years experience: With This Firm 1 With Other Firms	
e. Education: Degree(s)/Year/Specialization A.S./1986 Data Processing; B.S. , Johnson State University, 2000	
f. Active Registration: Year First Registered / Discipline	
g. Other Experience and Qualifications relevant to the proposed project: Responsible for identifying freshwater zooplankton 1999-2000: Sampled, identified (to species), and enumerated 180 zooplankton samples as part of a common loon study generated by myself and sponsored by Johnson State College.	

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APPENDIX B:
LABORATORY WASTE STORAGE AND DISPOSAL

APPENDIX B: Laboratory Waste Storage and Disposal

1.0 Introduction

Aquatec Biological Sciences handles and disposes of hazardous waste following U.S. EPA Regulations in 40 CFR 262 and 268 and Vermont Department of Environmental Conservation Regulations in Chapter 7. Laboratory hazardous waste is generated at the facility listed below:

Facility: 273 Commerce Street
Williston, VT 05495
(802) 655-1203

U.S. EPA Generator ID:
VTP000009663

2.0 Category

Presently the laboratory facility generates five hazardous waste categories. The following Table 2.1 lists the categories:

Table 2.1 Waste Categories

Williston (VTP000009663)

Acid
Aqueous (non-regulated)
Mixed Solvent
Soil (non-regulated)
Vials

Employees shall label each 55-gallon drum before transferring material into it. Hazardous waste labels are provided by the hazardous waste treatment, storage and disposal facility (TSDF). Material class labels, such as "Flammable", shall be purchased from a separate vendor. The shipping name, hazard class, waste code and additional information pertinent to each of the above hazardous waste categories are listed on the shipping labels.

3.0 Personal Protective Equipment

Employees removing hazardous waste from the laboratory sections shall wear safety glasses, a laboratory coat and nitrile gloves. Additional protective equipment, such as a face shield and apron, shall be worn while transferring acidic and caustic solutions into drums. Respirators are available for use at the discretion of employees who have been approved.

4.0 Accumulation

Laboratory sections that generate and accumulate hazardous waste and non-regulated waste are listed in Table 4.1.

Table 4.1 Laboratory Sections and Corresponding Waste

<u>Laboratory Section</u>	<u>Category</u>	<u>Description</u>
Toxicology	Aqueous	non-regulated water samples
	Soil	non-regulated soil samples
	Sediments	non-regulated sediment samples
Sample Management	Aqueous	non-regulated water samples
	Soil	non-regulated soil samples
	Sediments	non-regulated sediment samples
Microbiology	Aqueous	non-regulated water samples

Biologists and technicians shall accumulate hazardous waste and non-regulated waste in containers located at their workstation or stored in sealed containers in laboratory refrigerators. Table 4.2 lists the appropriate container for each hazardous or non-regulated waste category.

Table 4.2 Hazardous waste categories with appropriate container.

<u>Laboratory Section</u>	<u>Category</u>	<u>Original Container</u>
Toxicology	Aqueous	one to five gallon plastic container
	Soil	0.5 gallon to five gallon plastic container
Sample Management	Aqueous	original sample container
	Soil	original sample container

Microbiology Aqueous original sample container

Each container used to accumulate waste at the workstations shall be labeled with at least the name of the waste category.

5.0 Short Term Storage

The transfer of hazardous waste and non-regulated waste from the laboratory will be coordinated with each Department Manager. Hazardous or non-regulated solid waste is transferred to 55-gallon steel drums stored in the garage. Transport original sample containers of non-regulated waste in boxes or on a lab cart when a sample holding time has expired. Transport all other full containers of hazardous waste and non-regulated waste from the work stations to the hazardous waste room in a secure transport device such as a rubber bottle carrier or corrosion resistant utility cart designed to contain spills or leaks.

Transfer hazardous waste and non-regulated waste into the appropriate 30 or 55-gallon steel drum. After transferring waste into a drum, secure the bung or cover in place to contain vapors. After each DOT 17E and DOT 6D drum is full, tighten the bung securely with the bung wrench. After each DOT 17H drum is full, place a gasket in the rim of the cover, place the cover over the top of the drum then secure the cover on top of the drum with a drum ring and bolt. Place a sign on the drum indicating that it is full.

6.0 Disposal

As needed, contact the U.S. EPA approved TSDF (currently APT at 893-8281) to schedule shipment of hazardous waste and non-regulated waste for disposal. Report to the TSDF the categories and corresponding number of drums that will be shipped. The TSDF will provide a uniform hazardous waste manifest for waste hauling and disposal documentation. Mail copies of the uniform hazardous waste manifest to the state agency responsible for regulating hazardous waste where the TSDF is located, and to the Vermont Department of Environmental Conservation at 103 South Main Street in Waterbury, Vermont. Order empty drums from the TSDF to replace the full drums at the time of waste shipment.

APPENDIX C: LABORATORY METHODOLOGIES

Laboratory Methodologies

<i>Method Number</i>	<i>Method Description</i>
<u>Ecology Laboratory</u>	
1002CIF	Phytoplankton units/L
10200H3-C	Chlorophyll a, corrected ug/L
10200H3-U	Chlorophyll a, uncorrected ug/L
350.1	Ammonia, colorimetric, phenate mg/L
353.2	Nitrate-nitrite, colorimetric, cadmium reduction mg/l
365.1	Orthophosphorous, colorimetric, Ascorbic acid mg/L
FIAGE	Fish Aging
FILL	Fillet
HOMOG	Tissue Homogenization
MACROID	Macroinvertebrate Enumeration & Identification
MNS001	Micronutrient analyses; suite 1
OTOGND	Otolith mounting and grinding
PHYTOID	Phytoplankton Identification
SEXDER	Fish Sex Determination
ZOOID	Zooplankton Identification
<u>Microbiology Laboratory</u>	
2540G	Percent Solids %
2540GM	Percent Moisture %
9020B3c	Water Suitability
9213D	E. coli CFU/100 ml
9215B	Heterotrophic Plate Count (PP) CFU/ml
9215C	Heterotrophic Plate Count (SP) CFU/ml
9215C-AN	Anaerobic Heterotrophic plate count CFU/ml
9215C-S	HPC on solid CFU/gm
9215D	Heterotrophic Plate Count (MF) CFU/ml
9221B	Total Coliform (MPN) Col/100 ml
9221B-S	Total Coliform (MPN) col/gm
9221E	Fecal Coliform (MPN) Col/100 ml
9221E-S	Fecal Coliform (MPN) on Solids MPN/g (dry)
9222B	Total Coliform (MF) Col/100 ml
9222D	Fecal Coliform (MF) Col/100 ml

Laboratory Methodologies

<i>Method Number</i>	<i>Method Description</i>
9230C-EN	Enterococcus Col/100 ml
9230C-FS	Fecal Streptococcus Col/100 ml
9240B	Iron Bacteria (qualitative)
9240D	Iron Bacteria (quantitative) Col/100ml
9240D2A	Bacteria, Sphaerotilus and Leptothrix Col/100ml
9240D2c	Gallionella IB culture P/A
9240D2D	Heteotrophic Iron Bacteria Col/100ml
AM2001	Microtox Screening %
AM50ST	Microscopic Exam
BAM1	Total Coliform CFU/gram
BAM2	E. coli CFU/gram
BAM2-L	E.coli CFU/ml
BAM3	Total Bacteria CFU/gram
BAM3-L	Total Bacteria CFU/100 ml
BAM4	Salmonella spp. P/A
D5660-96	Microtox Basic Toxicity Test IC50
ISOGRID1	Total Yeast and Mold Col/gram
ISOGRID2	Gram Negative Bacteria CFU/gram
ISOGRID3	Staph. aureus Col/gram

Toxicology Laboratory

100.1HA	Amphipod, Hyalella azteca 10 Day Survival and Growth Test for Sediments
100.2CT	Midge, Chironomus tentans 10-Day Survival and Growth Test for Sediments
100.3	Lumbriculus variegates Bioaccumulation Test for Sediments
100.4AA	Amphipod, Ampelisca abdita, 10 Day Survival in Sediment
100.4EE	Amphipod, Eohaustorius estuarius, 10 Day Survival and Reburial Test
100.4HA42	Amphipod, Hyalella azteca, 42-day Survival, Growth and Reproduction Test
100.4HA28	Amphipod, Hyalella azteca, 28-day Survival, Growth Test

Laboratory Methodologies

Method Number	Method Description
100.4LP	Amphipod, Leptocheirus plumulosus, 10 Day Survival in Sediment
100.4RA	Amphipod, Rhepoxynius abronius, 10 Day Survival in Sediment
100.5CT28	Midge, Chironomus tentans 28-Day Survival and Emergence Test for Sediments
*1000.0	Fathead Minnow, Pimephales promelas, Larval Survival and Growth
*1001.0	Fathead minnow, Pimephales promelas, larval survival and teratogenicity
*1000.0SF	Salmonid Chronic 10 day Survival and Growth
*1002.0	Daphnid, Ceriodaphnia dubia, Survival and Reproduction
1003.0	Green Algae, Selenastrum capricornutum, Growth
*1004.0	Sheepshead Minnow, Cyprinodon variegatus, Larval Survival and Growth
*1005.0	Sheepshead Minnow, Cyprinodon variegatus, Embryo-larval Survival and Teratogenicity
*1006.0	Inland Silverside, Menidia beryllina, Larval Survival and Growth
*1007.0	Mysid, Americamysis bahia, Survival, Growth and Fecundity
*1008.0	Sea Urchin, Arbacia punctulata, Fertilization
*1009.0	Red Macroalga, Champia parvula, Reproduction
2340C	Hardness, EDTA Titrimetric mg as CaCO ₃ /L
A24CDR	Daphnid, Ceriodaphnia dubia 24 H Renewal Acute
A24CDS	Daphnid, Ceriodaphnia dubia 24 H Static Acute
A24CVR	Sheepshead Minnow, Cyprinodon variegatus 24 H Renewal Acute Test
A24CVS	Sheepshead Minnow, Cyprinodon variegatus 24 H Static Acute Test
A24DMR	Daphnid, Daphnia magna 24 H Renewal Acute Test
A24DMS	Daphnid, Daphnia magna 24 H Static Acute Test
A24DPR	Daphnid, Daphnia pulex 24 H Renewal Acute Test
A24DPS	Daphnid, Daphnia pulex 24 H Static Acute Test
A24MBR	Mysid, Americamysis bahia 24 H Renewal Acute Test
A24MBS	Mysid, Americamysis bahia 24 H Static Acute Test
A24MEBR	Silverside, Menidia beryllina 24 H Renewal Acute Test
A24MEBS	Silverside, Menidia beryllina 24 H Static Acute Test
A24PPR	Fathead Minnow, Pimephales promelas 24 H Renewal Acute Test
A24PPS	Fathead Minnow, Pimephales promelas 24 H Static Acute Test
*A48CDR	Daphnid, Ceriodaphnia dubia 48 H Renewal Acute

Laboratory Methodologies

<i>Method Number</i>	<i>Method Description</i>
*A48CDS	Daphnid, Ceriodaphnia dubia 48 H Static Acute
*A48CVR	Sheepshead Minnow, Cyprinodon variegatus 48 H Renewal Acute Test
*A48CVS	Sheepshead Minnow, Cyprinodon variegatus 48 H Static Acute Test
*A48DMR	Daphnid, Daphnia magna 48 H Renewal Acute Test
*A48DMS	Daphnid, Daphnia magna 48 H Static Acute Test
*A48DPR	Daphnid, Daphnia pulex 48 H Renewal Acute Test
*A48DPS	Daphnid, Daphnia pulex 48 H Static Acute Test
*A48MBR	Mysid, Americamysis bahia 48 H Renewal Acute Test
*A48MBS	Mysid, Americamysis bahia 48 H Static Acute Test
*A48MEBR	Silverside, Menidia beryllina 48 H Renewal Acute Test
*A48MEBS	Silverside, Menidia beryllina 48 H Static Acute Test
*A48PPR	Fathead Minnow, Pimephales promelas 48 H Renewal Acute Test
*A48PPS	Fathead Minnow, Pimephales promelas 48 H Static Acute Test
*A96CDR	Daphnid, Ceriodaphnia dubia 96 H Renewal Acute
*A96CDS	Daphnid, Ceriodaphnia dubia 96 H Static Acute
*A96CVR	Sheepshead Minnow, Cyprinodon variegatus 96 H Renewal Acute Test
*A96CVS	Sheepshead Minnow, Cyprinodon variegatus 96 H Static Acute Test
*A96DMR	Daphnid, Daphnia magna 96 H Renewal Acute Test
*A96DMS	Daphnid, Daphnia magna 96 H Static Acute Test
*A96DPR	Daphnid, Daphnia pulex 96 H Renewal Acute Test
*A96DPS	Daphnid, Daphnia pulex 96 H Static Acute Test
*A96MBR	Mysid, Mysidopsis bahia 96 H Renewal Acute Test
*A96MBS	Mysid, Americamysis bahia 96 H Static Acute Test
*A96MEBR	Silverside, Menidia beryllina 96 H Renewal Acute Test
*A96MEBS	Silverside, Menidia beryllina 96 H Static Acute Test
*A96PPR	Fathead Minnow, Pimephales promelas 96 H Renewal Acute Test

Laboratory Methodologies

<i>Method Number</i>	<i>Method Description</i>
*A96PPS	Fathead Minnow, Pimephales promelas 96 H Static Acute Test
ALK&HARD	Alkalinity & Hardness
B28MN	Macoma Clam, Macoma nasuta, 28-Day Sediment Bioaccumulation Test
C48MES	Blue Mussel, Mytilus edulis, 48 H Embryo-Larval Development
C72DES	Sand Dollar, Dendraster excentricus, 72 Hour Larval Development
C72SPS	Purple Urchin, Strongylocentrotus purpuratus 72 Hour Larval Development Urchins
CAHAZ22	Fathead Minnow, Pimphales promelas, Title 22 Hazardous Waste 96 H Acute
E1562-94	Polychaete, Neanthes arenaceodentata, 20-Day Survival and Growth Test
1000 Trout	Salmonid 10-day Survival and Growth Test

* NELAC accredited analysis.

APPENDIX D:
Certifications and Accreditations

CERTIFICATIONS

Aquatec is currently certified for microbiological analyses in Vermont. The following list is a summary of the professional certifications currently held for biological analyses.

Vermont Department of Health Laboratory

Certified for Bacteria in Drinking Water;

- Multiple tube fermentation, total coliform, method 9221B

- Membrane filtration, total coliform, method 9222B

Contact Person: Mr. W. George Mills

DMR

Toxicology

Aquatec participates in the annual USEPA DMR Whole Effluent Toxicity (WET) Quality Assurance Program. The program is currently administered by a private firm. For the 2004 and 2005 programs (DMR-QA Study 24 and Study 25 WET), Aquatec contracted with Environmental Resource Employees of Arvada, Colorado for reference standards and reporting.

Microbiology

Aquatec participates in bi-annual Proficiency Testing Program. Aquatec contracts with Environmental Resource Employees of Arvada, Colorado for reference standards and reporting.

ACCREDITATION

Aquatec is NELAP-Accredited laboratory for toxicity testing and microbiology analyses. Primary accreditation was awarded through the State of New Hampshire Environmental Laboratory Accreditation Program, in accordance with NELAC Standards (Certificate Number: 173704).

APPENDIX E: Figures

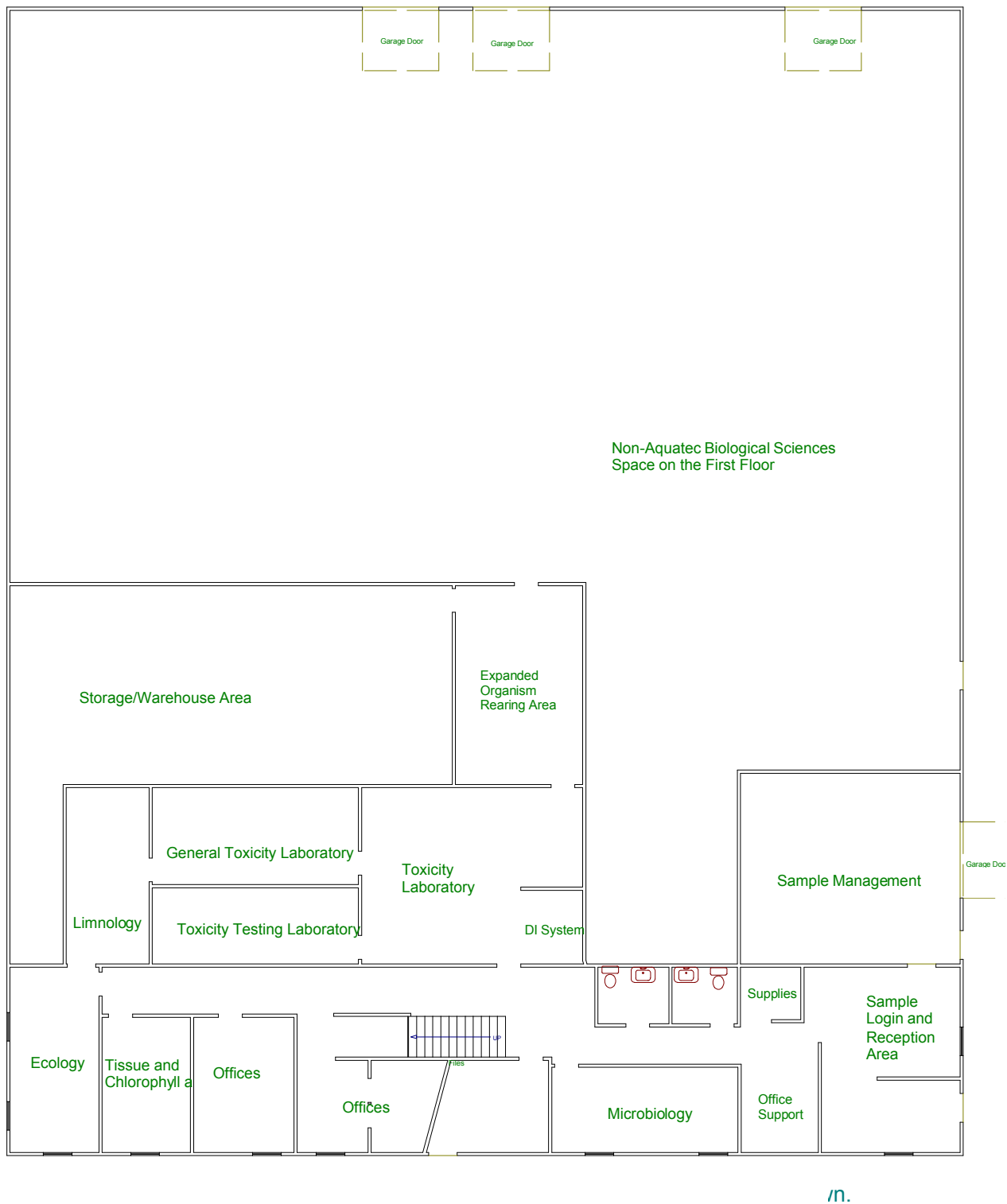


Figure 1. Floor plan for Aquatec Biological Sciences (Second Floor not Shown)

Demonstration of Capability

Laboratory Name: Aquatec Biological Sciences, Inc.

Analyst's Name (s):

Initial training: ☐

Continued proficiency evaluation: ☐

Department:

Ecology: ☐

Microbiology: ☐

Toxicology: ☒

Matrix: ☐ Water ☐ Sediment ☐ Soil Other (describe)

Method: (SOP, Revision, measured parameter):

SOP/Method:
Date read and agreement to follow:
Verbal description / demonstration completed?
Training performed by:
Training assessment (attach):

- 1) The analyst(s) identified above, using the cited test method(s) which is in use at this facility for the analyses of samples under the National Environmental Laboratory Accreditation Program, have met the Demonstration of Capability.
- 2) The test method(s) was performed by the analyst(s) identified on this form.
- 3) Raw data (including a copy of this form) necessary to reconstruct and validate these analyses are attached.

Technical Director: John Williams

Date:

QA Officer: Philip Downey, Ph.D.

Date:

Figure 2. Demonstration of Capability

AQUATEC BIOLOGICAL SCIENCES, INC.
RECORD OF INDIVIDUAL TRAINING

Week Ending: _____

Individual employees are to submit this form with their weekly time cards to substantiate all hours charged to Training & Education or Safety Training. Copies of all certificates, diplomas, transcripts or other documentation should be attached.

Payroll ID: _____ Employee Name (Print): _____ Division: _____

Project #: _____ Title: _____

THE ABOVE NAMED PERFORMED TRAINING
OR INSTRUCTION ON THE DATES AND FOR THE HOURS INDICATED.

Date	Hours	Nature of Duties, Training or Instruction	Code

I hereby certify that I have completed and understand the training listed above.

Employee Signature / Date

Trainer or Supervisor Signature / Date

Figure 3. Record of Individual Training

Record of Equipment Maintenance, Damage, Malfunction, and Repair

[illegible]

Figure 4. Equipment Maintenance and Repair

Corrective Action Report

Date: _____

Contract: _____

Initiated by: _____

BTR: _____

SDG: _____

Summary of Problem:

Summary of Investigation and Findings:

Resolution/Recommended Corrective Action:

Date of Implementation: _____

Follow-Up

Date: _____

Follow-Up by: _____

Follow-Up Findings:

Figure 5. Corrective Action Report

**AQUATEC BIOLOGICAL SCIENCES, INC.
CLIENT COMPLAINT INFORMATION**

DATE OF COMPLAINT:

PERSON RECEIVING COMPLAINT:

CLIENT / PERSON REGISTERING COMPLAINT

METHOD OF COMMUNICATION:

Phone: ☐

E-mail: ☐

Other:

DISCRIPTION OF COMPLAINT

ACTIONS TO BE TAKEN TO RESOLVE COMPLAINT

FOLLOW-UP INFORMATION / RESOLUTION

SUBMIT TO DEPARTMENT MANAGER AND DIRECTOR

SIGNATURE / DATE:

Figure 6. Client Complaint Form

Aquatec Biological Sciences

Chain-of-Custody Record

273 Commerce Street
Williston, VT 05495
TEL: (802) 860-1638
FAX: (802) 658-3189

COMPANY INFORMATION		COMPANY'S PROJECT INFORMATION			SHIPPING INFORMATION		VOLUME/CONTAINER TYPE/ PRESERVATIVE (NOTE 4)					
Name: _____		Project Name: _____			Carrier: _____		—	—	—	—	—	—
Address: _____		Project Number: _____			Airbill Number: _____							
Telephone: _____		Sampler Name(s): _____			Date Shipped: _____		—	—	—	—	—	—
Facsimile: _____		Quote #: _____ Client Code: _____			Hand Delivered: Q Yes Q No							
Contact Name: _____												

SAMPLE IDENTIFICATION (NOTE 1)	COLLECTION		GRAB	COMPOSITE	MATRIX	ANALYSIS/REMARKS (NOTE 2,3)	NUMBER OF CONTAINERS						
	DATE	TIME											

Relinquished by: <i>(signature)</i>	DATE	TIME	Received by: <i>(signature)</i>	NOTES TO SAMPLER(S): (1) Limit Sample Identification to 30 characters, if possible; (2) Indicate designated Lab Q.C. sample and type (e.g.:MS/MSD/REP) and provide sufficient sample; (3) Field duplicates are separate sample; (4) e.g.: 40 ml/glass/H ₂ SO ₄ Notes to Lab: _____ _____ _____
Relinquished by: <i>(signature)</i>	DATE	TIME	Received by: <i>(signature)</i>	
Relinquished by: <i>(signature)</i>	DATE	TIME	Received by: <i>(signature)</i>	

Distribution: Original Accompanies Shipment; Copy to Coordinator Field Files

Figure 8. Generic Chain-of-Custody Form

Aquatec Biological Sciences

273 Commerce

Chain-of-Custody Record

Street

Winston, VT

COMPANY INFORMATION		COMPANY'S PROJECT INFORMATION			SHIPPING INFORMATION		VOLUME/CONTAINER TYPE/ PRESERVATIVE						
Name: Client A Address: <u>222 Pleasant Street</u> <u>Suite 102</u> City/State/Zip: A town in, MA 01xxx Telephone: () Facsimile: Contact Name: Ms. Contact		Project Name: Outfall Composite Project Number: 06xxx Sampler Name(s): Quote #: Client Code: xxxx			Carrier: Airbill Number: Date Shipped: Hand Delivered: <input type="checkbox"/> Yes <input type="checkbox"/> No		4°C — Plas tic — 1	4°C — Plas tic — 1/2	4°C — Plas tic — 1 l	4°C — Glas s — 40	4°C — Amb erGl — 250	4°C — Plas tic — 0.5	
SAMPLE IDENTIFICATION		COLLECTION		GRAB	COMPO SITE	MATRIX	ANALYSIS (detection limits, mg/L)	NUMBER OF CONTAINERS					
		DATE	TIME										
Outfall Composite						Effluent	<i>Daphnia pulex</i> 48-h Static Acute Toxicity (EPA Method 2021.0). Log in for A48DPS	1					
Outfall Composite						Effluent	Total Residual Chlorine					1	
Pristine River						Receiv ng	Dilution Water	1					
Pristine River						Receiv ng	Total Residual Chlorine					1	
Relinquished by: (signature)		DATE	TIME	Received by: (signature)			NOTES TO SAMPLER(S): (1): Complete the labels (Date, time, initials) and cover the labels with clear tape. Tape the caps of the sample bottles to ensure that they do not become dislodged during shipment. Nest the samples in sufficient ice to maintain 0°C – 6°C. Results for samples received at temperatures exceeding 6°C will be qualified in the report. Notes to Lab: Ambient cooler temperature: °C. Dechlorinate the effluent sample if chlorine is detected.						
Relinquished by: (signature)		DATE	TIME	Received by: (signature)									
Relinquished by: (signature)		DATE	TIME	Received by: (signature)									

Figure 9. Project-specific Chain-of-Custody Form

**APPENDIX F
MASTER LIST OF EQUIPMENT**

**(NOTE: ADDITIONAL INFORMATION AND TRACKING OF MAINTENANCE
AND REPAIRS IS MAINTAINED IN THE ACCESS DATA BASE FOR EQUIPMENT.)**

Not attached electronically -Available upon request

**Standard Operating Procedure
for
Amphipod, *Hyalella azteca*, 28-day Survival and Growth
Toxicity Test for Sediments**

1.0 OBJECTIVE

This SOP describes procedures for performing a 28-day whole sediment survival and growth toxicity test. This test is used to estimate the chronic toxicity of whole sediment samples to the freshwater amphipod, *Hyalella azteca*. End points measured include survival and growth on Day 28. When required, toxicity is estimated by statistical comparisons of survival and growth (dry weight) data, to the organism responses in the control or reference site sediment. This procedure is a modification of the guidelines of EPA/600/R-99/064: *Methods for Assessing the Toxicity of and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* Second Edition, Method 100.4. In this modification the test duration is shortened from 42 days to 28 days by excluding the water-only phase exposure (Days 28-42) outlined in Method 100.4 and the reproduction endpoint is not determined.

WARNING: Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling these samples.

2.0 PREPARATION

Equipment and Apparatus

Calibrated Instrumentation and Water Quality Apparatus:

- pH meter
- Dissolved Oxygen (DO) meter
- Thermometer (accurate to 0.1°C), ScanLink temperature monitoring system
- Alkalinity and hardness titration apparatus
- Ammonia selective electrode and meter
- Mettler M3 Microbalance
- VWR 1320 drying oven

Additional Equipment:

- Test chambers (300-ml beakers, 8 per sample)
- Aeration manifold, tubing, manifold, and pipets
- Automated water-delivery system
- Disposable polyethylene transfer pipets
- Light tables
- Waste collection bucket
- Carolina bowls
- Nitex mesh sieves (0.5 mm)

Reagents:

- Reconstituted moderately hardwater (EPA/600/R-94/024)
- Deionized water
- 70 percent Ethanol

Forms and Paperwork:

- Amphipod (*Hyalella azteca*) Water Chemistry Data
- Amphipod (*Hyalella azteca*) Daily Biological Monitoring
- Amphipod (*Hyalella azteca*) Day 28 Survival and Growth Data
- Sediment Characterization Data
- Organism Holding and Acclimation
- Daily Checklist for Automated Delivery System
- Project Documentation Forms

Test System and Conditions

The test system and environmental conditions for the 28-day survival and growth test are summarized in Figure 1.

Test Organisms

Procurement and Documentation

Amphipods are obtained from a commercial supplier or from in-house cultures. When possible, schedule delivery of amphipods at least 24 hours prior to test initiation. They are acclimated to the exposure water used in testing during the the period prior to test initiation.

Prior to the testing, order sufficient organisms for 10 amphipods per replicate test chamber (80 per test sample) and a surplus for reference toxicant testing. Request that the supplier provide information regarding the age and environmental conditions for the test organisms.

Amphipods are shipped by next-day carrier and delivered to Aquatec Biological Sciences. Upon receipt, examine the organisms and document their apparent condition and the dissolved oxygen (D.O.), pH, temperature and conductivity of the shipping water. Record the observations on the Organism Data Sheet provided by the supplier. Place a copy of this sheet in the project data package.

Evaluation of Amphipod Condition

If, during examination, it appears that more than 5% of the organisms have died during transport, or if the temperature or other environmental conditions are widely different from test requirements notify the Toxicity Laboratory Manager immediately. A decision will be made regarding the possibility of obtaining a new stock of organisms for testing. If the test is to be delayed, document the reason. Also, it may be necessary to notify the client.

Acclimation and Holding

Transfer the amphipods to a 8-L plastic storage container. Add incremental amounts of laboratory reconstituted water and acclimate to test temperature (23°C). Provide aeration to the holding container. Overlying water temperature should not be changed more than 3°C per day. Monitor organism mortality, temperature, pH, D.O. during the holding period and record the monitoring data on the Organism Holding and Acclimation form. Amphipods should be 7-8 days old when the test is started. If more than five percent of the organisms die during the holding period, contact the Laboratory Manager and arrange for a replacement order.

Food

Feed daily sufficient *Selenastrum* and YCT to maintain a monolayer of food on the bottom of the container.

Exposure Water

Reconstituted moderately hardwater prepared following the procedure outlined in Section 7.1.3 of EPA/600/R-94/024 mixed 1:1 with natural water (e.g., Lake Champlain, Vermont) is used as exposure water (overlying water) during the test. Age the exposure water with vigorous aeration for at least one day prior to use in toxicity testing.

3.0 PROCEDURES

Control Sediment Preparation

Control sediment is formulated sediment prepared according to the procedure outlined in EPA/600/R-94/024 (Section 7.2.3.2) and consists of 77% fine and medium sand, 17% kaolinite clay, 5% ground peat, and 1% calcium carbonate. The formulated sediment is stored dry and is hydrated by addition of reconstituted moderately hardwater prior to distribution to test chambers.

Test Sediment Preparation

1. Remove sediment samples from sample storage refrigerators.
2. Transfer the sample to the the Sample Preparation Laboratory;
3. Homogenize the sediment with a clean plastic spatula;
4. Transfer aliquots of the homogenized sediment to a glass tray and examine for indigenous organisms;
5. If no indigenous organisms are apparent (check very carefully for amphipods), transfer approximately 100 mL aliquots to each of the replicate test chambers;
6. If indigenous organisms (especially predacious insects or amphipods) are present, remove them with forceps or press sieve sediment through a 1.0 mm Nitex mesh sieve, re-homogenize, and then distribute 100-mL aliquots to each of the test replicates. Notify the Laboratory Manager before making a decision to sieve sediments. Sieving of sediments should be avoided if possible;
7. Record the visual characteristics of each sediment sample on the Sediment Characterization Data form;
8. Add overlying water to a final volume of approximately 275 mL;
9. Return the unused sediment sample to Sample Management for storage;

10. Transfer the test chambers to the automated water delivery system and begin the water renewal cycles (e.g., noon and midnight). The test replicates remain in the test system overnight without addition of test organisms.

Measuring Initial Overlying Water Chemistry

On the day of test initiation (Day 0), remove an aliquot of overlying water from at least one replicate of each test sample. Measure the following parameters: pH, DO, and conductivity. Record the data directly on the Monitoring Data Form for Day 0. Aliquots of overlying water are also stored and preserved for Day 0 alkalinity, hardness, and ammonia analyses. The temperature of the exposure water should be within the range of $23 \pm 1^\circ\text{C}$. D. O. should be ≥ 2.5 mg/L. Additional water exchanges or aeration may be required if D.O. levels do not remain above 2.5 mg/L.. Temperature is measured by the automated temperature recording system (ScanLink)

Test Initiation: Preparation and Distribution of Test Organisms

1. Place the amphipod holding container over a light table and use a disposable polyethylene transfer pipet to transfer amphipods to 1-oz. (30 mL) disposable cups (Dixie condiment cups) until each cup contains 10 amphipods. Prepare sufficient cups for one per test replicate plus several spares. Sufficient amphipods (60) should be reserved for a standard reference toxicant test and for initial dry weight determination. Randomly select a cup containing 10 amphipods. Examine them over a light table and replace any apparently unhealthy or injured amphipods. The test replicates are positioned randomly within the testing system.
2. Gently rinse the 10 amphipods into a test replicate with clean exposure water using a transfer pipet. Check to be sure that all amphipods have been removed from the cup and swim to the sediment in the test replicate. A drop of exposure water can be used to submerge any amphipods that get trapped on the water surface.
WARNING: Do not dip condiment cups into the exposure water.
3. Record the date and time of test initiation when amphipods have been distributed to all test chambers.
4. After one hour, check all test replicates and replace any amphipods which are floating or are dead.
5. Preserve a representative sample of 8 cups containing 10 amphipods with 70% ethanol for measurement of initial lengths.

Daily Monitoring

Environmental Conditions

The environmental conditions monitoring schedule and list of parameters is outlined in Table 1. On Days 0 and 27 preserve a portion of the overlying water sample used for water quality determinations (approximately 100 mL) with 0.3 mL of concentrated H_2SO_4 for ammonia-N analysis and collect subsamples of overlying water for alkalinity and hardness analyses. These samples should be properly labeled and stored in a refrigerator at 4°C for subsequent analysis.

Biological Monitoring

Examine test beakers daily and record any observations of unusual organism behavior.

Feeding

Provide 1.0 mL of YCT to each test replicate daily. If the D.O. drops below 2.5 mg/L due to the accumulation of uneaten food, feeding may be suspended for 1-2 days. Document these events and increase the water renewal frequency (or aerate), if needed, to maintain acceptable D.O levels.

Automated Water Delivery System

Complete the System Checklist during the noon delivery cycle daily. Ensure that all components of the delivery system are functioning properly. Check the ScanLink temperature monitoring system at least at the beginning and end of each day.

End-point Determination

Day 28 Survival (8 Replicates)

1. Transfer each test replicate to a light table equipped with side lighting. Search for amphipods and remove both live or dead amphipods with a transfer pipet. Decant the overlying water through a 0.5 mm sieve. Rinse the sediment through a 0.5 mm sieve. Pool all surviving amphipods from a single replicate into a 30-mL disposable cup. Count and record the total number of surviving amphipods observed on the Survival Data Form. If organisms appear to be dead, examine them under a dissecting microscope. If any movement is detected, the amphipod is considered to be alive.
2. If fewer than 8 amphipods are recovered, transfer all sediment and material that has not passed through the 0.5 mm sieve back into the test chamber and hold the replicates for a possible reexamination. The test material may be preserved with sugar formalin solution and Rose-Bengal Stain and stored for a subsequent re-pick. Stained amphipods found during the repick will be assumed to have been alive on Day 28 if the body tissue is not significantly degraded. The total number surviving will then be corrected.

Day 28 Growth (8 Replicates)

The replicate cups containing surviving amphipods are processed for the Day 28 growth analysis. Growth is based upon the mean dry weight of pooled surviving amphipods, for each replicate. Transfer surviving amphipods to pre-weighed weighing boats (boat weights recorded on the Amphipod (*Hyalella azteca*) Day 28 Survival and Growth Data form) and dry overnight in the drying oven at approximately 60°C. Weigh the dried amphipods to the nearest 0.01 mg. The Mettler M3 microbalance is used for all dry weight determinations.

4.0 QUALITY ASSURANCE

Blind Sample Analysis

Each sample, including the Control, will be assigned a unique sample number which will be used throughout the test.

Test Acceptability

Test acceptability criteria are based upon the guidelines of EPA/600/R-99/064, Table 14.3 (excluding the reproduction requirements). Specifically, a test is judged to be acceptable if the average survival of control amphipods is equal to or greater than 80% on Day 28. The environmental conditions must be within the tolerance limits of *Hyalella azteca*.

Protocol Deviations

Any deviations from this SOP should be noted on a project documentation form and the Laboratory Manager and/or the Project Director should be immediately notified. The Project Director will determine the appropriate corrective action and will communicate protocol deviations to the client.

Reference Toxicant Testing

A water-only 96-hour exposure of amphipods to potassium chloride (KCl) will be conducted concurrently with the sediment exposures and with the same batch of amphipods. The 96-hour LC50 from this standard reference toxicant test is used to assess the sensitivity of the test organisms and to develop a control chart of LC50 values for this species when exposed to potassium chloride.

5.0 SAFETY

Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling these samples.

6.0 TRAINING

To be qualified for the overall procedure outlined in this SOP, the analyst must:

Read this SOP.

Receive verbal and visual instruction.

Demonstrate at least 90% recovery of 10 amphipods in sediments.

Be trained on pertinent associated SOPs.

Figure 1. Test conditions for the amphipod (*Hyalella azteca*) 28-day whole sediment chronic toxicity test.
ASSOCIATED PROTOCOLS: EPA 2000. *Methods for Assessing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Second Ed. (EPA/600/R-99/064) Method 100.4 (modified).

1. Test type:	Whole-sediment toxicity with renewal of overlying water
2. Temperature:	Average 23 ± 1 °C; instantaneous 23 ± 3 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illumination:	About 100 to 1000 lux
5. Photoperiod:	16 h light; 8 h dark
6. Test chamber size:	300 ml beaker
7. Sediment volume:	100 ml
8. Overlying water volume:	175 ml
9. Renewal of overlying water:	Two volume additions per day
10. Life stage of organisms:	Juveniles 7-8 days old
11. Organisms / test chamber:	10
12. Replicates / sample:	8
13. Feeding:	1.0 ml YCT daily per replicate test chamber
14. Aeration:	None, unless D.O. drops below 2.5 mg/L). Additional overlying water renewals may be preferable to aeration.
15. Overlying water:	Reconstituted moderately hard water and natural water (1:1)
16. Control sediment:	Formulated sediment
17. Sediment preparation:	Homogenize sediments before distribution to test chambers. Remove any indigenous organisms noted. If required, press sieve (1 mm sieve).
18. Water quality monitoring:	Daily: temperature 3 times weekly: Dissolved oxygen, pH Weekly: Conductivity Days 0 and 27: alkalinity, hardness, and ammonia
19. Biological monitoring:	Daily: Organism behavior
20. Test duration:	28 days
21. Endpoints:	Day 28: survival and growth (average dry weight)
22. Reference toxicant:	Potassium chloride, 96-h acute, water only
23. Test acceptability (control performance):	Minimum mean survival of 80% on Day 28
24. Data interpretation:	Hypothesis tests versus the control or the reference site responses

NEW HAMPSHIRE ENVIRONMENTAL LABORATORY ACCREDITATION PROGRAM

Aquatec Biological Sciences
Analyte List Number: 173705-A

273 Commerce Street Williston, VT 05495 (802) 860-1638
Date of Issue: July 6, 2005

Page 1 of 1
Expiration Date: July 5, 2006

GRANTS PRIMARY ACCREDITATION TO THE ABOVE MENTIONED LABORATORY FOR THE FOLLOWING ANALYSES:

Drinking Water Microbiology

Total Coliform	MF	SM 9222 B
Fecal Coliform	MTF	SM 9221 F

Non-Potable Water Microbiology

E. coli	MF	EPA 1103.1
Fecal Coliform	MTF	SM 9221 F

Toxicity Testing

Fresh Water Acute

Ceriodaphnia dubia	EPA/600/4-90/027F
Daphnia magna	EPA/600/4-90/027F
Daphnia pulex	EPA/600/4-90/027F
Oncorhynchus mykiss	EPA/600/4-90/027F
Pimephales promelas	EPA/600/4-90/027F
Salvelinus fontinalis	EPA/600/4-90/027F

Salt Water Acute

Cyprinodon variegatus	EPA/600/4-90/027F
Menidia beryllina	EPA/600/4-90/027F
Mysidopsis bahia	EPA/600/4-90/027F

Fresh Water Chronic

Ceriodaphnia dubia	EPA 1002
Pimephales promelas	EPA 1000
Pimephales promelas	EPA 1001

Salt Water Chronic

Arbacia punctulata	EPA 1008
Champia parvula	EPA 1009
Cyprinodon variegatus	EPA 1004
Menidia beryllina	EPA 1006
Mysidopsis bahia	EPA 1007

This analyte list supercedes all previously issued analyte lists.

Method accreditation does not imply acceptance for NHDES compliance testing.

Laboratory is required to use EPA approved methods where required by regulation.


Program Manager



*State of New Hampshire
Environmental Laboratory Accreditation Program*

Awards Primary Accreditation to

*Aquatec Biological Sciences
of
Williston, VT*

For the analyses listed on the attached page(s) in accordance with
the provisions of the NELAC Standards and Env-C 300.



Certificate Number: 173705

Date of Issue: July 6, 2005

Expiration Date: July 5, 2006

Bill Hall

Program Manager

Method accreditation does not imply acceptance for NHDES compliance testing.
Laboratory is required to use EPA approved methods where required by regulation.
Continuing accreditation status is dependent on successful ongoing participation in the program.
Customers may verify the laboratory's current accreditation status by calling (603) 271-2998.

NEW HAMPSHIRE ENVIRONMENTAL LABORATORY ACCREDITATION PROGRAM

Aquatec Biological Sciences
Analyte List Number: 173705-A

273 Commerce Street Williston, VT 05495 (802) 860-1638
Date of Issue: July 6, 2005

Page 1 of 1
Expiration Date: July 5, 2006

GRANTS PRIMARY ACCREDITATION TO THE ABOVE MENTIONED LABORATORY FOR THE FOLLOWING ANALYSES:

Drinking Water Microbiology

Total Coliform	MF	SM 9222 B
Fecal Coliform	MTF	SM 9221 F

Non-Potable Water Microbiology

E. coli	MF	EPA 1103.1
Fecal Coliform	MTF	SM 9221 F

Toxicity Testing

Fresh Water Acute

Ceriodaphnia dubia	EPA/600/4-90/027F
Daphnia magna	EPA/600/4-90/027F
Daphnia pulex	EPA/600/4-90/027F
Oncorhynchus mykiss	EPA/600/4-90/027F
Pimephales promelas	EPA/600/4-90/027F
Salvelinus fontinalis	EPA/600/4-90/027F

Salt Water Acute

Cyprinodon variegatus	EPA/600/4-90/027F
Menidia beryllina	EPA/600/4-90/027F
Mysidopsis bahia	EPA/600/4-90/027F

Fresh Water Chronic

Ceriodaphnia dubia	EPA 1002
Pimephales promelas	EPA 1000
Pimephales promelas	EPA 1001

Salt Water Chronic

Arbacia punctulata	EPA 1008
Champia parvula	EPA 1009
Cyprinodon variegatus	EPA 1004
Menidia beryllina	EPA 1006
Mysidopsis bahia	EPA 1007

This analyte list supercedes all previously issued analyte lists.

Method accreditation does not imply acceptance for NHDES compliance testing.

Laboratory is required to use EPA approved methods where required by regulation.

Bill Hall

Program Manager

